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PROCEEDINGS OF NORTHWEST SHELLFISH SANITATION RESEARCH PLANNING CONFERENCE (1964)

W. J. Beck, et al

Northwest Shellfish Sanitation Laboratory
Gig Harbor, Washington

1965

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Northwest Shellfish Sanitation Research Planning Conference

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U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
Public Health Service

1964
PROCEEDINGS

Northwest Shellfish Sanitation Research Planning Conference

W.J. Beck, J.C. Hoff and T.H. Ericksen

Northwest Shellfish Sanitation Laboratory
Gig Harbor, Washington 98335

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U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
Division of Environmental Engineering and Food Protection
Shellfish Sanitation Branch
1965

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PREFACE

In 1959 the first Annual Northwest Shellfish Sanitation Research Planning Conference was held at Purdy, Washington. The principal objectives of research in problems relating to the sanitary control of shellfish in the Pacific Northwest were outlined at this time.

Many portions of the original objectives have been accomplished. However, as one facet of research has been completed other projects in the same realm remain to be completed. Thus many of the basic objectives of the original conference are appropriate six years later. New problems have emerged in the ensuing years that have required expanding the scope of our research activities beyond that outlined in the plans developed in 1959. Publication of proceedings of the 1964 conference will inform participants in the cooperative program of progress being made in attacking these more recent problems.

We are deeply grateful to the conferees for their frank discussions of the problems through the past years. Participation by personnel of State and Federal agencies, both in this country and Canada, universities and industry has made these conferences successful. We pledge this laboratory to continue in the spirit of cooperation that has prevailed in all phases of sanitary control of shellfish on the international level.

W. J. Beck

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Summarization of Activities

W. J. Beck

During the past few years several parts of the original proposed program of research activities of 1959 have been completed. The initial stage of research dealing with methods of analysis and fate of indicator organisms as shown in the ecological study is ready for publication. Various studies on storage of shellfish are reported in these proceedings. The laboratory phase of accumulation-elimination of bacteria by shellfish has been completed.

With the phasing out of certain research projects others have been initiated as proposed by the conferees at the Annual Planning Conference. One set of data in oceanography of a local estuary has been obtained. This study was initiated to complement the sanitary and bacteriological surveys made in the past. Accumulation-elimination studies of viruses by shellfish have also been initiated.

Through the cooperation of Washington State Departments of Health and Fisheries, samples of Pacific oysters and overlying water were analyzed for DDT-DDE during the Hemlock Looper spraying program on Willapa Basin.

Technical assistance has been given to State and Regional offices. The laboratory evaluation of the West Coast States has been placed on an annual schedule. A paper on fecal coliform in shellfish growing areas has been prepared for presentation at the forthcoming National Shellfish Sanitation Workshop. Several short-term training courses for in-service and State Laborator, personnel have been conducted on various aspects of shellfish sanitation bacteriology.

The year has been one of change and expansion. As one research project was completed other aspects of the overall problem in the sanitary control of shellfish remained to be solved. With addition of new personnel and more space we hope to delve more deeply into the many complex problems associated with shellfish sanitation research.

Research Progress Reports

A Study of the Applicability of Several Indices as Sanitary Quality Indicators in Commercially Packed Pacific Oysters (Crassostrea gigas)

J. C. Hoff, W. J. Beck and M. W. Presnell

INTRODUCTION

Various tests used in other areas of sanitary bacteriology have been used to determine the microbiological quality of fresh packed oysters in commercial channels. The most important objective of such a test is to determine the disease transmitting potential of the product. Therefore, such a test, or tests, should indicate whether or not the shellfish have been exposed to fecal pollution from man and other warm blooded animals and the degree of such exposure if present. Such a test or index should remain stable during proper marketing operations subsequent to harvesting, thereby indicating the sanitary quality of the product as harvested and reflecting potentially hazardous mishandling during processing.

Another objective, also related to public health, is to determine the freshness, i.e. presence or absence of spoilage of the product. Since temperature and duration of holding time are two primary factors influencing spoilage, such a test, or tests, should reflect the quality of handling from these two aspects. It is obvious that the same test could not satisfy both objectives, since in the first instance the index should remain stable and in the second the index should change in response to temperature and time.

This study was undertaken to investigate the value of various indices relative to the above objectives and also to determine whether or not modification of the present maximum temperature recommended for handling of shucked shellfish would be advisable. The investigation was initiated in January, 1961 and continued through June, 1963. Modifications in storage temperatures and bacterial indices applied were made when predicated by results obtained.

MATERIALS AND METHODS

Description of Study:

In the 1961 studies, the behavior of five groups of indicator organisms in Pacific oysters (Crassostrea gigas) during storage at 3 C and -21 C was determined. Individual experiments were conducted over a period of several months to evaluate possible seasonal effects on the index groups. The index groups studied included the coliform, fecal coliform, fecal streptococcus, 35 C standard plate count, and 20 C plate count in sea water agar. The pH of samples was also determined. The results of this phase of the study indicated that the fecal coliform test and the 35 C standard plate count gave the best indication of quality. Little change in any of the indices occurred at -21 C.

Accordingly, in the 1962-63 studies, the -21 C storage temperature was discontinued. In these studies, oysters were stored in crushed ice and at 3 C and 10 C dry storage. The latter temperature is the upper limit recommended for storage of shucked shellfish (Jensen, 1962). Studies of the coliform, fecal coliform, and 35 C plate count index groups and pH determinations were continued in the 1962-63 studies. Commercial sources were broadened to include some Oregon plants and lots were collected through several seasons as before.

Oysters:

The oysters used in this study were obtained from commercial sources in Washington and Oregon. Each lot consisted of 36 commercially packed and sealed twelve-ounce containers. The containers were collected immediately after shucking, washing and packing. They were then placed in refrigerator cases in crushed ice, and transported to the laboratory. Transport time varied from a few minutes to eight hours, depending on the proximity of the commercial source. On arrival at the laboratory each lot was subdivided for storage at various temperatures.

Iced samples were stored in insulated chests and kept constantly and completely surrounded by crushed ice. Standard refrigerators equipped with special thermoregulators and facilities for air circulation were used for dry storage at 3 C and 10 C. One container from each lot, sampled immediately on arrival at the laboratory, provided the 0 hour sample for all three temperatures.

Samples and Sampling Schedule:

The samples consisted of the entire contents of one twelve-ounce container after one oyster had been removed aseptically and chopped for the pH determination. The sampling schedules for the three storage temperatures were as follows:

(1) Iced Storage: 0 hour, 1,2,4,7,10,15,20 and 25 days.

(2) 3 C Storage: Same as (1) except sampling terminated on 20th day.

(3) 10 C Storage: 0 hour, 1,2,3,4,5 and 6 days.

These periods were sufficiently long so that definite signs of spoilage, indicated by off-odor and cell lysis, were apparent at or before the last sampling time at each temperature.

Examination:

The bacteriological indices determined on samples consisted of five tube coliform and fecal coliform MPN's and standard plate counts at 35 C performed according to recommended procedures (1962). The pH was determined electrometrically. Results of examination for fecal streptococci and 20 C plate counts in sea water agar and the results of -21 C storage are not included because these tests were discontinued in the latter part of the study.

In some of the experiments, IMViC tests on cultures isolated from EC positive tubes were performed to determine whether or not a differential die-off of Escherichia coli occurred during storage.

Storage schedule:

Storage experiments were planned so that at least three lots were examined in each of three seasons: summer, winter, and spring. In this way, possible effects of seasonal variations in the oysters and their bacterial flora on changes in bacterial densities in the shucked product could be evaluated.

RESULTS

The chronology of the storage experiments and the classification of the data for analysis are shown in Table 1. In the 1961 studies changes in some of the indices in the summer lots differed from those observed in the winter and spring lots. Therefore, these lots were separated into two groups. Since analysis of the 1962-63 results indicated that similar bacteriological changes occurred in all lots, these were placed in one group. Table 1 shows that the summer lots generally were collected following period of much less rainfall than the winter and spring lots. This difference was more pronounced in 1961 than in 1962-63.

Geometric mean values for coliform MPN's and 35 C plate counts in each group were calculated. Because some fecal coliform MPN's were indeterminate (<18), median values were calculated for this index. Arithmetic mean values were used for the pH data. In most of the 1961 studies, duplicate determinations were performed on each sample. In the 1962-63 studies, determinations were done singly. In a few instances, due to laboratory accidents or indeterminate results, data were not available for a particular sampling time. In some lots, storage was terminated earlier than in others because of definite signs of spoilage. In these instances, the results are based on fewer than the number of lots indicated. The results are shown in Figures 1-5.

A comparison of the behavior of coliform MPN's and 35 C plate counts in 1961 winter-spring and summer lots stored at 3 C is shown in Fig. 1. The summer lots contained lower initial levels of both indices. Also, the initiation of rapid multiplication of both groups was delayed in the summer lots. Coliform MPN's and 35 C plate counts in the summer lots remained at their initial levels for 3 days and 5 days longer, respectively, than in the winter-spring lots. Coliform MPN's in the 1961 lots increased slowly and in one case appeared to decline after the tenth day of storage. This may not have been an actual reduction since there was evidence of inhibition in the lower MPN dilutions. In some cases gas was not produced in the lower dilutions in the presumptive medium but was produced in higher dilutions. In other instances, inocula transferred from positive lower dilution tubes failed to produce gas in Brilliant Green Bile Broth while growth transferred from positive higher dilution tubes did produce gas. However, the patterns of gas positive tubes were so erratic that it was not possible to interpret the data on the basis of definite inhibition. Results similar to these were encountered in a few individual samples from several subsequent lots. Plate count densities were not noticeably affected by the inhibition.

The changes in coliform MPN's of the 1962-63 lots stored in ice, 3 C and 10 C are shown in Fig. 2. The behavior of coliform MPN's in these lots stored at 3 C differed from those observed in the 1961 lots. Rapid multiplication of coliforms in the 1962-63 lots began immediately and numbers became progressively higher. Coliform MPN's in iced samples showed little initial stability but increased less rapidly than at 3 C. At 10 C, coliform MPN's began to increase immediately and rapidly, reaching very high levels by the fourth day of storage.

The changes in 35 C plate counts in the 1962-63 lots stored in ice, 3 C and 10 C are shown in Fig. 3. At 3 C, the 35 C plate counts in the 1962-63 lots began to increase sooner than in the 1961 lots (see Fig.1). The 35 C plate counts in the 1962-63 summer lots stored at 3 C increased much more rapidly than in the 1961 summer lots. In iced samples, the 35 C plate count remained relatively stable for 7 days, then increased rather rapidly. At 10 C the 35 C plate counts increased very rapidly, reaching high levels by the fourth day of storage.

Changes in fecal coliform MPN's during storage are shown in Fig. 4 (1961 lots) and Fig. 5 (1962-63 lots). As indicated above these results are based on median values rather than geometric means. At 3 C and in ice, fecal coliform MPN's remained quite stable but decreased somewhat during the storage period. At 10 C (Fig. 5) fecal coliform MPN's did increase about thirtyfold during the storage period of six days. This was much lower than the increases in coliform MPN's and 35 C plate counts.

The results of IMViC tests on isolates from EC positive tubes are shown in Table 2. The number of isolates is limited since IMViC tests were performed only at selected intervals. No evidence of either selective survival or dieoff of E. coli compared with other IMViC types is evident.

The results of pH determinations are shown in Table 3. Since differences in pH changes between the winter-spring lots and the summer lots collected in 1962-63 were apparent, these results are presented separately. The largest pH decline in the 1961 lots occurred in the winter-spring lots during the first day of storage. In the 1963 summer lots the greatest decline occurred during the first day of storage at all three temperatures. In the case of 3 C and ice storage, these sudden drops were not correlated with increases in coliform, fecal coliform or plate count densities. In addition, in most cases, similar pH values at all three temperatures were shown after two and four days storage. However, coliform and plate count densities were much higher in oysters stored at 10 C than at the two lower temperatures at these time intervals (Figs. 2 and 3).

DISCUSSION

The data presented above confirm and extend the observation of Presnell, (1962) regarding the fecal coliform group enumerated by the EC test. That is, that this group ... "showed the most consistent patterns of change and was influenced least by duration or condition of storage or by season." A similar observation was made as a result of storage studies on the Eastern oyster (Crassostrea virginica) on the Gulf Coast by Presnell and Kelly (1961). Their storage studies were carried out at 2-12 C for as long as 23 days.

Fecal coliform densities did increase somewhat during storage at 10 C. However, the rapid increases in coliform and 35 C plate count densities at this temperature and rapid deterioration of the oysters indicated that 10 C is not an acceptable storage temperature for shucked oysters. Fecal coliform densities decreased slightly during storage at 3 C and in ice. However, in view of the great increases in coliform and 35 C plate count densities with consequent production of substances potentially toxic to fecal coliforms, the stability of the group of these temperatures was remarkable.

Since fecal coliform densities increased at 10 C, it would be reasonable to assume that enteric pathogens might also multiply during storage at this temperature. Presnell and Kelly (1961) showed that E. coli and a mixed suspension of Salmonella derby, Salmonella infantis, and and Salmonella newport showed similar patterns of increase in Eastern oysters stored at 20 C. Similarly, one might postulate that numbers of

enteric pathogens might be reduced during storage at 3 C and in ice. In neither case is there direct evidence from the current studies to support these conjectures. Kelly and Arcisz (1954) found that E. coli and Salmonella schottmuelleri showed similar changes in numbers in shell oysters (Crassostrea virginica) and soft clams (Mya arenaria) stored at 5 C and 19 C. At both temperatures in both shellfish species similar reductions in numbers occurred. However, in shell oysters stored at 5 C the rate of reduction of S. schottmuelleri was less than that of E. coli in the initial period of storage.

Evidence for the biological soundness of the use of fecal coliforms as an indicator of fecal pollution by man and other warm blooded animals has been given by Kelly (1960), Presnell, (1961), Kelly et al,(1962), Beck et al (1963) and Kabler et al (1964). Because of this, and because of the stability of this index during storage indicated above, it appears that the organisms enumerated by the EC test would be of value as an indicator of the sanitary quality of shucked oysters during the marketable life of the product.

The 35 C plate count, because of its marked response to storage temperature differences would be useful as an indicator of temperatures maintained and length of time involved in transport and holding shucked oysters in commercial channels. While differences in initial densities of microorganisms enumerated by this test and different patterns of increase were shown by different lots of oysters, the changes observed correlated overall with time and temperature of storage.

The coliform group has disadvantages as an indicator of either sanitary quality or state of freshness of the product. Large increases in density of this index occurred at all three temperatures, making it unsuitable as a stable indicator of the sanitary quality of shucked oysters when harvested. Changes in numbers in response to storage time and temperature were much less consistent than shown by the 35 C plate count index, making the coliform index less desirable as an index of shucked oyster spoilage.

The results of this study indicate that pH determinations were of little value in determining the quality of shucked Pacific oysters. A definite change toward lower pH levels was observed but the changes in many cases were not correlated with changes in any of the microbial indices used in this study. From some of the results above, it would appear that factors other than bacterial multiplication are major causes of postmortem pH changes in Pacific oysters.

It is evident from the results presented above that 10 C is not a satisfactory temperature for holding shucked Pacific oysters because of the rapid increase in microorganisms observed at this temperature. It is also evident that as one approaches 0 C, the rate of multiplication of microorganisms present in the oysters becomes much lower. The rates of enzymatic reactions causing autolysis are also temperature dependent. Therefore, it is obvious that storage temperatures which approach 0 C as nearly as possible will result in much longer shelf life for shucked Pacific oysters.

SUMMARY

A study of the applicability of coliform MPN, fecal coliform MPN, 35 C plate count, and pH as indicators of the quality of commercially packed Pacific oysters stored in crushed ice, and at 3 C and 10 C dry storage was made over a period of nearly three years.

The results indicated that the fecal coliform MPN, because of its stability during storage and its value as an indicator of fecal pollution, would be of value as an indicator of sanitary quality of the oysters when packed. The 35 C plate count, because of its response to storage conditions, i.e. time and temperature, appeared to be a satisfactory indicator of spoilage. Coliform MPN's were not stable and did not show consistent changes in all lots of oysters. The pH gave little information as to the bacteriological quality of the oysters.

As storage temperatures approached 0 C, rates of bacterial multiplication became slower and the lag before multiplication began became longer. Storage either in ice or at 3 C greatly extended the marketable life of the product beyond that observed during storage at 10 C.

COMMENTS BY PARTICIPANTS

Mr. Sam Reed opened the discussion by asking for comments. Mr. Neufeld discussed the relationship of indicator organisms. Dr. Hoff quoted earlier work by Kelly and Arcisz on the relationship of indicator organisms to pathogenic organisms in light of the results from the present paper. Mr. Kelly discussed the methods used and the relationship of Salmonellae to the indicator organisms used in the Gulf Coast study.

Dr. Liston commented on the relative fate of fecal coliform, coliform and total plate count at the various storage temperatures. Mr. Neufeld stated that somewhat similar results had been observed in British Columbia.

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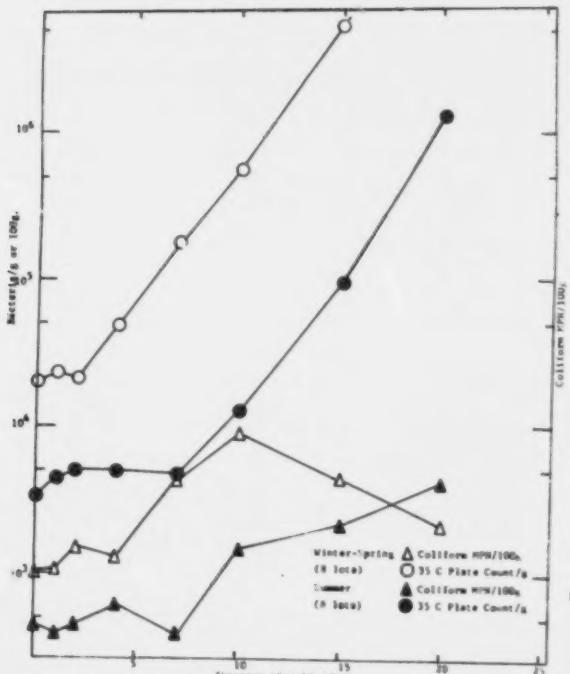

Fig. 1. Changes in coliform MPN and 35 C plate count in shucked Pacific oysters stored at 3 C (1961 lots)

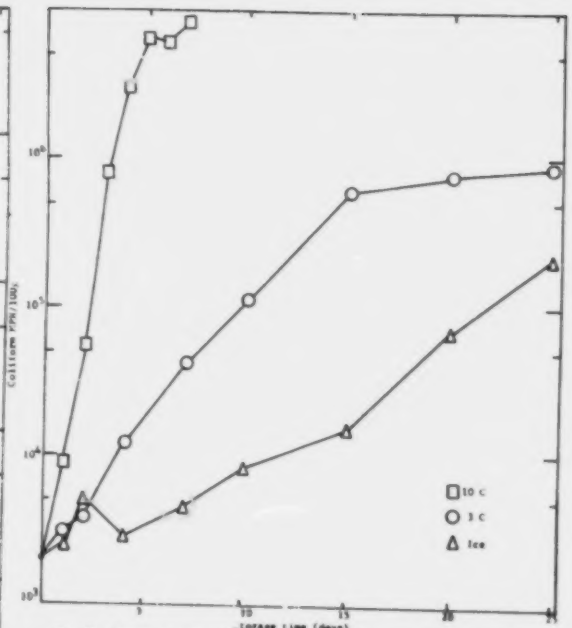

Fig. 2. Changes in coliform MPN in shucked Pacific oysters stored in ice, and at 3 C and 10 C (1962-63 lots)

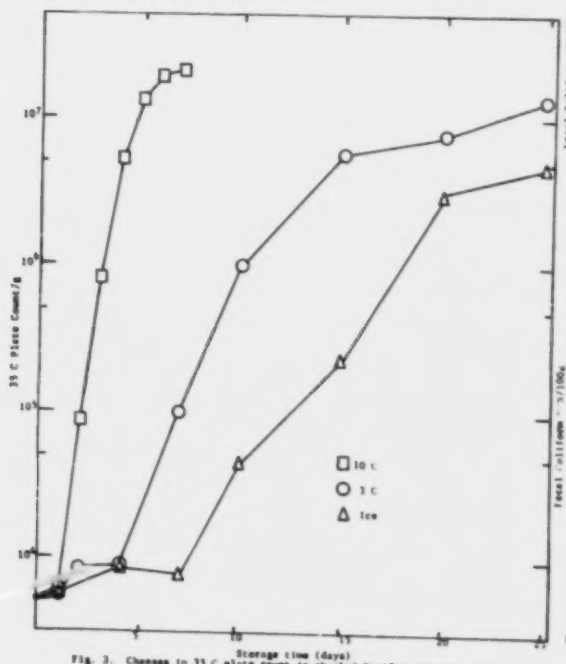

Fig. 3. Changes in 35 C plate count in shucked Pacific oysters stored in ice, and at 3 C and 10 C (1962-63 lots)

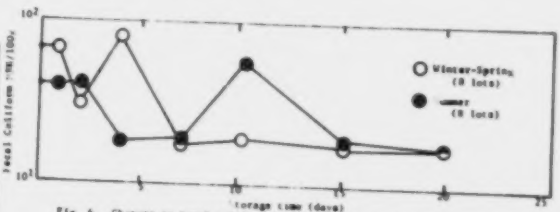

Fig. 4. Changes in fecal coliform MPN in shucked Pacific oysters stored at 3 C (1961 lots)

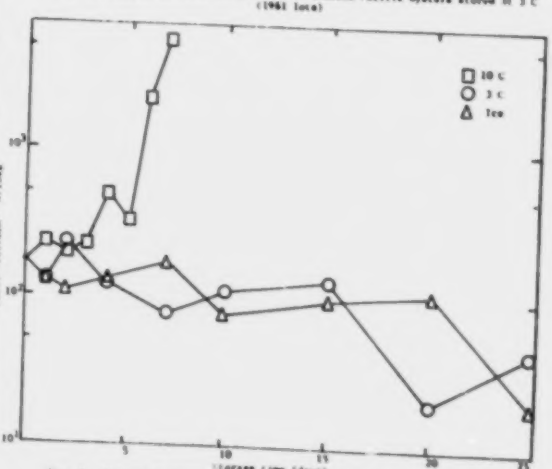

Fig. 5. Changes in fecal coliform MPN in shucked Pacific oysters stored in ice, and at 3 C and 10 C (1962-63 lots)

Table 1. Grouping of shucked Pacific oyster storage for data analysis

Storage Temperatures	Storage Classification	Lot No.	Date Storage Initiated	Inches Rainfall[a] in preceding 7 days	14 days
3 C	8 lots Winter-Spring	1	1-11-61	3.48	3.54
		2	1-12-61	3.50	3.56
		3	1-17-61	5.25	8.75
		4	1-17-61	5.25	8.75
		5	2-10-61	4.07	5.13
		6	2-10-61	4.07	5.13
		7	4-21-61	1.35	2.51
		8	4-21-61	1.35	2.51
	8 lots Summer, 1961	9	6-26-61	0.0	0.0
		10	6-27-61	0.0	0.0
		11	6-28-61	0.0	0.0
		12	8-15-61	0.0	0.0
		13	9-18-61	0.0	0.03
		14	9-18-61	0.0	0.03
		15	9-18-61	0.0	0.03
		16	9-18-61	0.0	0.03
Ice, 3 C and 10 C	15 lots Winter, Spring and Summer 1962-63	17	11-26-62	7.96	11.33
		18	11-26-62	7.96	11.33
		19	11-26-62	6.96	11.68
		20	3- 4-63	1.84	3.32
		21	3- 4-63	1.84	3.32
		22	3- 4-63	0.29	0.79
		23	4- 1-63	5.29	5.93
		24	4- 1-63	5.29	5.93
		25	4- 1-63	2.41	2.51
		26	4- 1-63	2.83	3.74
		27	6-10-63	0.64	0.84
		28	6-10-63	0.64	0.84
		29	6-10-63	0.64	1.27
		30	6-10-63	0.64	1.27
		31	6-10-63	0.44	0.56

[a]Data from: Climatological Data, Washington and Oregon. Volumes 65, 66 and 67 (1961,62 and 63), U. S. Dept. of Commerce, National Weather Records Center, Asheville, N. C.

Table 2. IMViC analysis of EC positive cultures isolated during storage of shucked Pacific oysters

Storage (Days)	Storage Temperature: Iced, 1962-63 lots, No. of Isolates	Iced, 1962-63 lots, % E.coli	3 C, 1961 lots[a], No. of Isolates	3 C, 1961 lots[a], % E.coli	3 C, 1962-63 lots, No. of Isolates	3 C, 1962-63 lots, % E.coli	10 C, 1962-63 lots, No. of Isolates	10 C, 1962-63 lots, % E.coli
0	43	74	39	92				
1	11	82	46	72	6	33	15	87
2			108	75				
3							16	100
4	33	80	63	84	33	80	74	88
5							13	92
6							16	100
7	8	63	60	82	8	88	18	78
10	31	93	60	83	29	93		
15	24	96	28	100	6	83		
20	28	90	25	64	4	100		
25	5	100	5	100				
30	24	100						

[a] 1961 data from Presnell, M. W, 1962. Studies on stored Pacific oysters (presented at Shellfish Sanitation Research Conference, Purdy, Washington).

Table 3. Changes in pH of shucked Pacific oysters stored at 10 C, 3 C and in ice

Time (Days)	Ice		3 C				10 C	
	1962-63 lots		1961 lots		1962-63 lots		1962-63 lots	
	Winter-Spring	Summer	Winter-Spring	Summer	Winter-Spring	Summer	Winter-Spring	Summer
0	6.4[a]	6.5	6.5	6.6	6.4	6.5	6.4	6.5
1	6.4	6.1	6.2	6.4	6.4	6.2	6.3	6.0
2	6.4	6.0	6.1	6.2	6.3	6.1	6.2	6.0
3							6.1	6.0
4	6.4	6.1	6.0	6.1	6.2	6.1	6.1	5.9
5							5.9	5.7
6							5.9	5.6
7	6.2	5.8	5.9	5.8	6.1	5.8		
10	6.1	5.9	5.7	5.6	6.0	5.8		
15	6.0	5.9	5.5	5.5	6.0	5.8		
20	6.0	5.9	5.7	5.6	5.9	6.0		
25	5.9	5.9				5.8		

[a]Arithmetic means of individual determinations.

Effect of Antifoaming Agents and Evacuation on Shellfish Homogenization

T. H. Ericksen

INTRODUCTION

It has been observed that during the blending process of the bacteriological examination of shellfish foaming occurred. Since the indices of bacterial density as described in APHA Recommended Procedures for the Bacteriological Examination of Sea Water and Shellfish (Third Edition, 1962), are based on a weight-to-volume relationship, any property which would interfere with this relationship may affect the number of bacteria indicated by these indices.

Preliminary data were obtained on the weights of 10 ml aliquots of the 1:2 dilution of shellfish homogenates. The ideal weight-to-volume relationship would be 10g/10ml. The results indicated this relationship was less than maximum. The average weight per 10 ml of these samples was 7.9g. It was believed that this deficiency was due to foaming during the blending process. Therefore, a number of companies were contacted and 17 different defoaming compounds were obtained. These compounds were examined and evaluated on the basis of toxicity, ability to reduce foaming and practicality. Also included was a method of blending under reduced atmospheric pressure.

MATERIALS AND METHODS

An aluminum Waring blendor jar was modified by laboratory personnel for attachment to a vacuum pump. It was also modified by the addition of aluminum baffling fins to insure homogeneous blending. Pacific oysters (Crassostrea gigas), Manila clams (Tapes japonica) and Native Littleneck clams (Protothaca staminea) were obtained from various sources to supply different bacterial populations for testing.

Each shellfish sample was shucked in the prescribed manner, then chopped and divided into 100g portions. These portions were treated in the following manner:

1. The control sample was blended with the shellfish meats and liquor only for 15-20 seconds, after which a 20 ml aliquot was removed and the pH determined. An equal weight of phosphate buffer was added to the sample and blending continued. After blending for the prescribed period, duplicate 10 ml aliquots were pipetted to tared beakers. A 20 ml aliquot was obtained for the pH determination and the bacteriological examination then proceeded as outlined in recommended procedures.

2. A minimum sample of 100g of shellfish meats and liquor was weighed and placed in a sterile modified blendor jar. An equal weight of phosphate buffer was added to the sample to be evacuated. The blendor jar was placed on the motor and attached to the vacuum pump. The container was evacuated to a vacuum gauge reading of 15 pounds. This mixture was blended for the prescribed time (90 seconds for oysters and 100 seconds for clams). After blending the atmosphere within the blendor container was allowed to reach equilibrium with that of the surrounding atmosphere very rapidly. Duplicate 10 ml aliquots were immediately pipetted to tared beakers. A 20 ml aliquot was obtained for the electrometric pH determination. Bacteriological examination then followed the outline in recommended procedures.

3. The antifoam compound was added to the phosphate buffer and sterilized. A shellfish sample was weighed and placed in a blending container. An equal weight of the phosphate buffer with the antifoam agent added was placed in the blending container. After blending for the prescribed period two 10 ml duplicate aliquots were pipetted to tared beakers, after which a 20 ml aliquot was obtained for the pH determination. The bacterial examination then followed as outlined in recommended procedures.

The pH determinations were obtained on the schedule as described above to determine the effects of evacuation or the antifoam agent on the pH of the shellfish homogenates.

RESULTS

Weights of twenty-one 10 ml preliminary samples of random shellfish homogenates diluted 1:2 with an equal weight of phosphate buffer and blended for 90 to 100 seconds ranged from 5.8g to 9.0g with an average of 7.9g. This data indicated that all dilutions contained approximately 20 percent less shellfish meats than the ideal amount.

All compounds investigated to date have been abandoned in favor of the evacuation method because of either their inability to be sterilized or to reduce foaming. Ten of these compounds were eliminated because of undesirable solubility and emulsifying properties. The remaining seven compounds were eliminated on the basis of inability to reduce foaming. However, one compound which showed some promise was tested further.

The weight-to-volume relationships of the control, evacuation method and antifoam agent are shown in Table 1. These values are based on 18 Pacific oyster samples. These data indicated that the evacuation method gave the most consistent maximum values.

Data on the effect of evacuation and the antifoam agent on the coliform MPN, fecal coliform MPN and 35 C plate count are given in Table 2. The data were analyzed by three different methods; median, geometric mean and probability plot.

The control values of the coliform MPN for the three methods of analysis ranged from 2200 to 2400. The values for the evacuation method ranged from 1800 to 2700 and the values for the antifoam agent ranged from 1500 to 1900. Fecal coliform MPN values ranged from 94 to 96 for the control; 73 to 97 for the evacuation method, and 120 to 130 for the antifoam agent. The 35 C plate count values ranged from 3900 to 5000 for the control; 4300 to 5200 for the evacuation method, and 2900 to 3600 for the antifoam agent.

The most distinct differences are found in the 35 C plate count values for the three methods of analysis.

Electrometric pH determinations on the homogenates of the evacuated samples, antifoam agent samples and control samples indicated no effect on pH because of the method employed.

In addition to the above a few samples of Pacific Coast hardshell clam species have been examined. However, sufficient data were not available for analysis at the time.

DISCUSSION

From the limited data presented it appears that a source of error in bacteriological examination of shellfish may exist. The use of antifoam agents has not proven practical to date. The method of choice may be that of evacuation technics.

The possibility of obtainiug and using glass jars for evacuation has been discussed. However, recent correspondence with Dr. Thomas Hosty, Director, Bureau of Laboratories, Dept. of Public Health, State of Alabama, has indicated that suitable glass containers may not be available. Further inquiries with glass container manufacturers will be made as to possible availability of appropriate containers.

This investigation will be continued. Emphasis will be placed on obtaining readily available, inexpensive evacuation blending containers and determining the effects of lower vacuum pressures on foam reduction and bacterial densities.

COMMENTS BY PARTICIPANTS

Dr. Dollar discussed the use of other antifoaming agents that might be available as well as other methods of homogenization. Dr. Liston questioned the limits that should be placed on present methods of

homogenization of shellfish samples. Dr. Liston questioned whether or not the margin of error shown would have significant bearing on the final results due to other inherent fluctuations in technics used. It was generally agreed that further investigation must be made before this could be resolved.

Table 1. Weights of duplicate 10 ml aliquots of Pacific oyster homogenates with two foam control technics

	Weight in grams of 10 ml aliquots		
	Control	Evacuation	Antifoam Agent
Range	6.7 - 9.1	8.0 - 10	7.0 - 9.9
Average	8.3	9.5	8.9

Table 2. Bacterial densities of Pacific oysters with two foam control technics

Method of Analysis	Bacterial Densities								
	Coliform MPN			Fecal Coliform MPN			35 C Plate Count		
	Foam Control			Foam Control			Foam Control		
	None	Evacuation	Antifoam Agent	None	Evacuation	Antifoam Agent	None	Evacuation	Antifoam Agent
Median	2400	2700	1700	94	73	130	5000	5200	3500
Geometric Mean	2200	1800	1900	98	97	120	3900	4300	3600
Probability Percentila									
10	34	21	120	20	16	40	250	410	430
50	2300	2300	1500	96	97	120	3400	5000	2900
90	16000	23000	18000	450	590	340	45000	61000	20000

Activities of Public Health Service Research Centers

C. B. Kelly[1]

In 1962, Congress appropriated more than $1,500,000 for constructing and equipping two shellfish sanitation research centers. The Gulf Coast Shellfish Sanitation Research Center, located at Dauphin Island, Alabama, completed July 1963, has a proposed staff of some 32 scientists and supporting staff, in a facility of approximately 10,000 square feet in area. The Northeast Center, some 20,000 square feet in area located at Narragansett, Rhode Island was occupied in May 1964. Both laboratories are similar in design and are equally complete in facilities, including laboratories for chemistry, biochemistry, microbiology, marine microbiology, virology, and marine biology. Each has an experimental laboratory with flowing sea water where in vivo experiments with shellfish can be conducted.

A unique feature of each Center is a Field Investigations Unit that serves two purposes; it gives technical assistance to the Regions, States, and Industry in problems of unusual nature and it augments the staff of the Regional Offices, assisting them in the evaluation of State shellfish sanitation control programs. The Field Investigations Unit provides engineers and other scientists acting as a buffer to the research scientists, allowing an uninterrupted program of research to continue. Technical assistance activities of the Northeast Shellfish Sanitation Research Center included collaboration with Maine, Rhode Island and Massachusetts in the design, development, and evaluation of pilot facilities for depuration of shellfish, a cooperative study with Maryland, New York, and New York City in commercial handling of soft clams, assistance to Maine in the development of sanitary survey of two shellfish areas, investigations in Connecticut and New Jersey in the incidents of hepatitis due to clams. At Dauphin Island, the projects included a cooperative study with Alabama to evaluate the proposed criteria for shellfish areas and shellfish at the market, assistance to Florida in shellfish toxicity in the Sarasota-Tampa Bay area, and assistance to Louisiana in pesticides in shellfish areas.

Of paramount importance in this day of rapid urbanization of coastal areas is the need for knowledge and development of technology for depuration. In spite of an active program in pollution control, shellfish control authorities have progressively found it necessary to prohibit harvesting of shellfish from many productive areas to

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protect the public from dangers of chance contamination due to mechanical failures in waste treatment systems. A system of decontamination of shellfish comparable in principle to the pasteurization of milk would afford the needed public health protection to utilize shellfish from these marginal areas. That shellfish will cleanse themselves is a well-recognized biological principle. The need is for the development of technology that would transcribe research knowledge to commercial application. A three phase program of research in depuration is in progress. Research knowledge is now available to demonstrate the effectiveness of the feeding-cleansing process in all species of shellfish. Highly efficient systems have been developed for the sterilization of sea water. The second phase, now initiated, will develop a practical process for depuration, first in pilot plants, then in commercial size facilities. Long term research needs will gather basic knowledge of the physiological processes that govern the accumulation, retention, and elimination of bacteria, viruses and toxic chemicals. Ultimate goals are to develop a system effective for all noxious materials and at an economically realistic level.

The Centers are fully equipped for research in virology. The importance of such research came to focus in 1961 when some 80 cases of infectious hepatitis occurred in Mississippi and Alabama traced to the consumption of raw oysters, the source being a localized area at the mouth of a heavily contaminated river. Later, hepatitis was associated with the consumption of raw clams in New Jersey and Pennsylvania. Although the exact source was not determined, the polluted areas in Raritan Bay were strongly incriminated. While the epidemiology of these outbreaks does not present evidence of failure of the current technical standards, that they occurred certainly demonstrated the need for basic investigations in the fate of viruses in the marine environment and their behavior in shellfish and the development of effective means for decontamination.

Following the massive fish kill in the Mississippi River last March, a united effort was exerted to determine the potential hazard of such agricultural chemicals to consumers of shellfish. The Public Health Service laboratories at Cincinnati and Dauphin Island collaborated in the determination of the cause of the mortalities and the Dauphin Island Laboratory conducted surveys in oyster growing areas. These studies are still in progress. A program of monitoring shellfish areas for toxic materials is in progress in representative shellfish growing areas in the Gulf Coast and South Atlantic areas.

The scientific literature for many years has mentioned the presence of certain substances in marine flora and fauna that inhibit the growth of viruses and bacteria. Of particular significance is the recent discovery of antimicrobial, antiviral and most recently tumor inhibiting agents from certain species of edible mollusks.

Such findings indicate the need for investigation of the presence of these substances in sea water and the flora and fauna of the marine environment. They are important as they relate to an understanding of the biological forces in the sea responsible for maintaining the biological balance and the destruction of organisms of terrestrial origin, but these biologically active materials might be of clinical significance.

Recent outbreaks of botulinum food poisoning from smoked fish brings to focus the need for investigations of toxigenic spore forming bacteria in the marine environment. While adequate preparation, primarily canning, of the commercially prepared product will prevent such occurrences, it is important to determine the distribution of such bacteria in the marine environment, the conditions under which the organisms can multiply and/or produce toxin in shellfish.

The need for studies of marine toxins has been recognized for many years. The Northeast Center will embark on studies of the ecology of the paralytic shellfish poison. Research will be conducted on the development of methods for the cultivation and growth of the causative organisms, the isolation and purification of the toxin, ultimately to gain a better understanding of the physiology and biochemistry of the paralytic shellfish poison. The Gulf Coast Shellfish Sanitation Research Center is conducting studies in the shellfish toxin found in the Tampa-Sarasota Bay area. Research and field investigations includes the monitoring of areas in the Gulf Coast for the presence of the toxin, isolation of toxins from shellfish and studies on the ecology of the organisms involved.

Shellfish sanitation research facilities in New England and the Gulf Coast cannot contribute research information completely of value to the West Coast industry and control agencies, because of different climatic and environmental characteristics and the difference in species of shellfish under commercial exploitation. To correct this research weakness, the Public Health Service is planning for the construction of a Northwest Shellfish Research Center, similar in design to the two existing facilities which will provide for a multi-disciplinary effort, much broader in scope than now possible at Purdy, to work on problems unique to the West Coast industry. Facilities for virology, chemistry, biochemistry, microbiology, biology and oceanography will provide for a team approach in fundamental research. An engineering and technical assistance staff will assist, consult and advise control components in the Regions and States and to industry in shellfish sanitation problems. We look toward the construction of this facility within the next five years. We look forward to this as a further accomplishment of our program responsibilities.

Brief Hydrographic Survey of Burley Lagoon, Washington

Philip S. Kelley[1]

INTRODUCTION

In many of its shellfish sanitation projects, the Shellfish Sanitation Branch has a need for oceanographic data to supplement the bacteriological and sanitary surveys usually taken. Since much, if not all, of the work presently contemplated will be confined to estuarine waters where shellfish are found, it seemed desirable to evaluate available methods of oceanographic sampling in such estuarine waters. To pursue this goal, it was decided to undertake a limited hydrographic survey of a small tidal estuary.

The estuary studied was Burley Lagoon, upon the shore of which is located the Northwest Shellfish Sanitation Laboratory. Burley Lagoon (Fig. 1) is located at the head of Carr Inlet, a large arm of southern Puget Sound in the State of Washington. It is a narrow, nearly flat-bottomed estuary, covering about 370 acres, and is oriented with its long axis almost exactly north-south. The lagoon is protected by a long spit across its southern end. There are dwellings on the shore almost everywhere except the spit. Oysters grow well in the lagoon, and a commercial harvesting operation based in part upon these oysters is quite active. Two streams contribute fresh water to the lagoon: Burley Creek at the head and Purdy Creek near the mouth of the lagoon. Both creeks flow year round and both have stream gages near their mouths which are read regularly. In addition, there are a few small seasonal streams which drain from the ridge to the west of the lagoon. These flow in the late fall and winter when precipitation is high.

The study of this lagoon was restricted to simple observations of temperature, salinity, currents and bottom topography. Limitations on personnel, equipment and time precluded any attempt to study the biological, chemical and geological conditions in the lagoon. Equipment had to be of such size and tractability as to allow its being handled without undue difficulty over the side of a sixteen-foot outboard motorboat, and it was desirable that the gear be simple enough that untrained personnel would have no difficulty using it in the future. A current meter and portable salinometer were obtained; these will be described in more detail below. Other necessary items were made in the laboratory as needed. The topography, the currents, and the temperature and salinity observations will be discussed separately in the paragraphs following.

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BATHYMETRY

Method:

Soundings were taken at times when there was sufficient water in the lagoon to float the boat. The 87 soundings recorded are listed in Table 1. The locations of the soundings, with the exceptions of numbers 81 and 82, are shown in Fig. 2. The two missing stations lie in Henderson Bay, just off the border of this chart south of station 80. Most of the soundings were taken with the boat moving slowly. The sounder consisted of a light line, marked at one foot intervals and weighted with a three-pound cast-iron sash weight. One observer cast the sounder, while the other guided the boat and recorded depths as the first called them out.

The soundings obtained were corrected for the stand of the tide at the time of observation and reduced to depths below mean higher high water at Wauna, Washington, which is on Henderson Bay at the west end of the spit across the lower end of Burley Lagoon. Tidal data and corrections for Wauna to be applied to Seattle predictions as published by the U. S. Coast and Geodetic Survey (1962) are shown in Table 2. The contour map based on the corrected soundings is shown in Fig. 3. The shapes of the contours have been checked and corrected somewhat to conform generally with the forms seen on stereoscopically viewed aerial photographs of the lagoon.

Discussion:

From Fig. 3 it can be seen that Burley Lagoon is a fairly shallow estuary, generally flat-bottomed except at the southern end. The area north of the island is covered with soft mud to a depth of several feet, whereas south of the island the bottom is fairly firm, being sandy or gravelly in places. The depths are such that on a moderately low tide (about a foot above mean lower low water) most of the lagoon above a line somewhere between the 12 and 15 foot contours shown will be dry and exposed. The deepest place found, a depth of 31 feet, was in the hole shown near the center of the lower half of the lagoon. Local residents claim that a fresh-water spring exists at the bottom of this hole, but evidence of this was not found, as will be shown later. The banks of the lagoon are quite steep. Because of this, the 3 and 6 foot contours are not shown on Fig. 3, but will be approximately equally spaced between the shoreline and the 9 foot contour.

A polar planimeter was used to measure the areas enclosed by each of the contour lines. These areas were substituted into the formula

given by Raisz (1948, p.112) $V = i\left(\frac{A_1}{2} + A_2 + A_3 + \ldots\ldots + A_n\right)$ where V is the volume of the body of water, i is the contour interval, A_1 is the surface area, and A_2, A_3 and so forth are the areas enclosed by consecutively lower contour lines. The approximate volume of Burley Lagoon estimated by this method is 1.9 x 108 cu. ft. or about 4,300 acre-ft., approximately 68% of which lies above the 9 foot contour. In making this calculation, the areas enclosed by the 3 and 6 foot contours were approximated by linear interpolation between the 0 and 9 foot contour areas. The volume of 4,300 acre-ft. is the amount of water the lagoon will hold at mean higher high water, or at a tide of 13.1 feet above mean lower low water.

The contour lines shown in Fig. 3 represent with reasonable accuracy the relative shape of the lagoon floor. However, there is evidence that the contours may be somewhat shallower than shown by perhaps a foot or so. The aerial photographs mentioned above were taken on 3 September 1963 at about 1:30 P.M. when the stand of the tide was almost exactly one foot above mean lower low water. These photographs show somewhat less water in the lagoon than would be predicted from the contour map and the stand of the tide at the time. Whether this discrepancy is the result of an actual systematic error in measurements and data reduction, or the result of a lag between the time of a given tide height at Wauna on Henderson Bay and the time of the same height inside the lagoon cannot be determined without further observations. Until the source of the discrepancy can be definitely located, the absolute values of the depths in, as well as the volume of, the lagoon must be considered as only rough approximations.

CURRENTS

Methods and Instruments:

Currents were measured with the boat anchored in position; stations where currents were measured are shown on Fig. 2. Except for stations D and E, it was found desirable to anchor the boat bow and stern to prevent undesirable swinging caused by shifting winds. At Stations D and E the current was strong enough during the time observations were made to obviate the necessity for a stern anchor. The boat was anchored from the bow by a standard Navy-type steel small-boat anchor and from the stern by two concrete building blocks totalling about thirty pounds in the water. The boat was anchored bow into the current.

The current meter used was the Model 622-SW-1 Price current meter, serial number 610356, manufactured by W. and L. E. Gurley of Troy, N.Y. This meter, together with its battery-operated electric counter, is shown in Fig. 4. A description of this type of meter, together with useful suggestions on handling and maintenance, is found in a publication by the U. S. Coast and Geodetic Survey: Manual of Current Observations, Special Publication No. 215 (1950, pp. 19-24). This meter is simple in construction and mode of operation. An electric circuit, consisting of a 6-volt dry cell battery in series with an electric counter and twin-wire rubber-insulated cable which also serves as a suspension cable for

the meter, is made and broken by a cam mounted on the upper end of the bucket wheel shaft. The cam closes the circuit momentarily once for each revolution of the bucket wheel, causing the counter to register each revolution of the wheel. The number of revolutions per minute of the wheel may be easily converted to current velocity in meters per second, feet per second or knots by means of rating tables, two of which are supplied by the manufacturer.

The meter was rigged off the port side of the boat, as pictured in Figs. 5 and 6. The suspension cable of the meter was market at one-foot intervals with paint, enabling easy positioning of the instrument at any desired depth, down to about 25 feet. A small boom, fashioned from two-by-four lumber and a clothesline pulley, was mounted on the port side. A cable clamp, made from an ordinary hasp and an eye-bolt and lined with thick rubber to prevent damage to the cable, was fastened to the boom. The boom was fastened by one bolt through the seat back, and could be either swung up and over or completely removed when the boat was being carried on a trailer.

The observer must be able to see the meter in order to know the direction of the current, as the Price design has no other means of indicating direction. It was found, using the two-conductor rubber-covered cable for suspension of the meter, that the stiffness of the suspension may be sufficient to hold the meter in an orientation not consistent with the direction of the current, especially if the meter was only a few feet down and/or the current was quite weak. The direction of the current was indicated by pieces of white string, several inches long, fastened to the cable just above the meter. After the meter was stabilized in the water it was possible by twisting the cable slightly where it passed over the pulley to cause the meter to orient itself into the current. Where the current was strong enough to exert sufficient force on the stabilizing vanes to orient the meter properly this procedure was unnecessary. It was also found essential to keep the contact chamber, which contains the cam and electrical contact, filled with light oil to exclude sea water. Any sea water in this chamber will create a short circuit, jamming the counter and causing corrosion in the contact chamber.

Located on the deck (to the right of the wheel, just above the ignition switch in Fig. 6) was a box containing a Navy Mark I four-inch boat compass, mounted in gimbals. The box may be rotated horizontally through 360°, and has two slots on opposite sides through which sightings for bearings may be made. The box is held to the boat by a single bolt and wing-nut, and may be easily removed. The compass itself may also be removed from the box. The true direction of the current was obtained by noting the orientation of the meter, lining up the marks on the compass frame approximately parallel to the meter, and adding 22° for variation to the reading.

Data was gathered at eight stations in Burley Lagoon and Henderson Bay during September and October, 1963. With two exceptions, the meter was suspended about halfway between surface and bottom. At station D, the current was strong enough to cause considerable vibration of the meter suspension cable, hence the meter was kept near the surface to reduce the vibration. Observations at station F on 15 October were made

both at the surface and midway down, because the velocities observed at the two depths differed substantially. This was the only time this effect was noticed. By "surface" is meant a depth of between one and two feet, deep enough to keep the meter below the level of the keel of the boat.

Discussion:

The currents measured generally showed little change in direction during the course of the ebb or flood on which they were measured. The notable exception was at station B on the ebb tide, where a complete reversal in direction occurred because of an eddy which developed as the water level fell. Figs. 7 and 8 show the direction and maximum velocities observed on the ebb and flood tides. The tidal range was not the same in all cases, varying from 12.2 to 5.1 feet, so the velocities are not always strictly comparable one to another.

Figs. 7 and 8 also show the general current pattern in the lagoon as observed qualitatively. One point is particularly worthy of note. This is the large, strong eddy, centered northwest of the power line towers standing in the southern end of the lagoon, which develops on the flood tide. As can be seen from Fig. 7 the current also flows south on the ebb tide. Hence this section of shore, along with the north shore of the spit, is affected by a unidirectional current, whereas the rest of the shoreline of the lagoon is influenced by a current which reverses itself every six hours. One effect of this current pattern is to cause some of any pollution introduced along the southwest shore the lagoon during flood tide to end up on the east shore, rather than the west shore, further up the lagoon. The current pattern also tends to ensure the stability of the sand spit as a natural feature.

With the exception of those flowing through the narrow mouth, the measured currents in the lagoon itself were fairly weak, always less than a knot, averaging overall about 0.25 knot. The maximum velocity observed at the mouth was 1.89 knots on a tidal change (flood tide) of 12.2 feet. Currents in excess of two knots are probably not uncommon in this vicinity. As noted above, at station F what appeared to be a definite velocity gradient between the surface and a depth of about 6 feet was observed. This was the only time and place that such an effect was pronounced enough to be obvious before lowering the meter. There was sufficient plankton in the water at most stations to enable the observer to compare velocities at the surface and at a depth of several feet before lowering the meter. In all cases except this one, there was no discernible difference, and hence the current meter was usually left at depth, with only occasional adjustments to correct for the changing level of the tide. The reason for the gradient observed at station F is not known.

TEMPERATURE AND SALINITY.

Methods and Instruments:

Salinity and temperature were measured at the same time and place that currents were. Observations were recorded about every fifteen minutes; data were generally taken from the surface as well as at the depth of the current meter. Some observations were recorded for the bottom, but these did not differ significantly from those halfway to the bottcm.

The instrument used for these observations was a Model RS5-2 Electrodeless Induction Salinometer, serial number 124, manufactured by Industrial Instruments, Inc., of Cedar Grove, New Jersey. This instrument, together with its probe, is shown in Fig. 9. The device measures salinity, temperature and conductivity in the ranges and accuracies shown:

Salinity: 0-40 o/oo ± 0.3 o/oo (temp. range 0-27 C)

Temperature: 0-40 C ± 0.5 C

Conductivity: 0-60 millimhos/cm ± 0.5 millimhos cm.

According to the manufacturer, accuracies may be improved by approximately one order of magnitude by the use of error curves.

The control box of the instrument contains an oscillator with an output of 5 volts at 2.3 kilocycles/second, a four-stage amplifier developing a gain of about 1000, a discriminator circuit with a gain of about 4, and a D. C. Wheatstone bridge circuit. The electronics are all solid-state and power is uppplied by nine 1.3 volt mercury cells. The transducer contains a thermistor head and two toroidal coils arranged about a hollow epoxy tube. The coils themselves are potted in epoxy and hence insulated from the salt water, but the hollow tube, open at both ends, allows sea water to form a core common to both toroids. The oscillator drives the first coil, which generates a closed alternating current loop in the sea water. The magnitude of this current is a function of the electrical conductivity of the water forming the core of the coil. The current loop in turn induces a current in the second toroid; the output of this coil is boosted by the amplifier. The discriminator compares the amplifier output with the oscillator signal, and causes the meter to deflect in the proper direction as dictated by the phase relationship of the two signals. The meter is then balanced to null by means of a manually adjusted slide-wire and two auxiliary coils, one of which is in the transducer. These coils together with the slide-wire are used to induce a flux in the second transducer coil opposite to that induced by the sea-water couple; when the fluxes are equal but opposite, the meter reads null. A linkage attached to the slide-wire shaft allows the conductivity to be read directly in the lower window of the control box.

The thermistor in the transducer is wired as one leg of the D. C. Wheatstone bridge circuit, and when the instrument is used to read temperature this circuit is all that is in use. When salinity is read, the resistance of the conductivity circuit is switched into another leg of the Wheatstone bridge, and a self-contained computor circuit integrates the two into a salinity value. Hence, to read salinity, conductivity must be measured first and the reading so obtained left undisturbed while salinity is being read. A four-position switch allows selection of the three described modes of operation of the instrument (conductivity, salinity and temperature) as well as a battery check.

The transducer is located at one end of 100 feet of five-conductor cable. The manufacturer cautions against subjecting the transducer to rough treatment such as dropping and further warns against using in currents in excess of two knots unless some strain relief is provided for the cable. Readings should not be taken when the transducer is less than six inches in any direction from any solid object, as such object will distort the induced current loop which in turn may lead to an erroneous reading. It was presumed that this restriction also applied to the air-water interface.

The salinometer cable was rigged off the starboard side of the boat using a boom arrangement identical to that used for the current meter. The cable was weighted with a three-pound weight, which was suspended about two feet below the transducer. The cable was marked with paint at one-foot intervals, allowing the transducer to be positioned at any depth down to about 95 feet.

Data and Discussion:

Readings were taken at the same time that current measurements were made. Readings were taken both at the surface and at various depths, with emphasis on the surface. Table 3 shows the average values of temperature and salinity by station for surface and subsurface observations.

The variations in salinity and temperature during the period of observation were slight. The weather during this time was generally clear and warm, with no rainfall and low runoff. The exceptions to this are reflected in the data for stations F and H. The effect of rainfall preceding the taking of data on the second occupation of these stations is seen in the somewhat lower surface salinities. Overall, however, there was little variation from the average temperature and salinity of about 13.7 C and 29.5 o/oo respectively.

These conditions of low runoff and rainfall do not persist through the fall and winter, and consequently no inferences about the effects of the runoff on the lagoon may be drawn for times other than those covered by the period of observations. As mentioned above, two creeks contribute runoff to the lagoon on a year-round basis. Of these, Burley Creek contributes the larger proportion. Table 4 shows approximate values for the

discharges of Burley and Purdy Creeks during September and October of 1963. These values were obtained from graphs relating stream gage height in feet to stream discharge in second-feet. The graphs were based on reduced Burley and Purdy Creek data for 1962 made available by the U.S. Geological Survey; the data for 1963 had not been processed by the Survey at the time of this writing. Since it is known that the relationships between gage height and discharge volume may very from year to year because of changes in the stream bed near the gage, the values given for runoff in Table 4 are only approximate. The months of November, December and January generally have considerably more precipitation than the months preceding, e.g., in December 1962 the average discharge from both Purdy and Burley Creeks was some 99 acre-feet per day as compared with 27.5 acre-feet per day for October, 1963. It is therefore quite apparent that further observations will be needed to examine the effects of runoff.

It was mentioned above that there might be another source of fresh water for the lagoon: a spring in the bottom of the hole in the center of the southern half of the lagoon. Certain local residents claim to have seen fresh water boiling to the surface or even geysering into the air from this position, especially following the last severe earthquake, presumably in 1949. The hole was investigated thoroughly with the salinometer but no significant variation from the average salinity was observed. The area surrounding Burley Lagoon is served by many drilled artesian wells, but no natural artesian water is known. Drilled wells in the vicinity reach artesian water below hard-rock at about 130 feet below sea level; water pressure at a sea-level wellhead is reported to be between ten and twenty pounds per square inch. Knowing this the possibility that the hole may be associated with artesian fresh water cannot be excluded. However, if such activity does exist, it must occur at irregular intervals, or some evidence of fresh water would have been found in the form of lower salinity or a slick on the surface near the hole.

GENERAL DISCUSSION

Methods:

The equipment and techniques described above satisfactory in many ways, but some limitations should be noted. The rig described and shown in Figs. 5 and 6 is quite obviously a fair weather arrangement. On Puget Sound there are sufficient winds during the late fall, winter, and spring that the Coast Guard posts small-craft warnings a large part of the time; working in these winds on the open sound with a sixteen-foot boat is out of the question. Even protected areas such as Burley Lagoon may become quite rough in a short period of time when the wind comes up quickly; during the seasons mentioned one cannot count on being able to spend much time anchored on a station.

Having reasonably calm weather is a necessity for making current measurements with the equipment described above. As can be readily seen from Fig. 4 the shape of the buckets on the bucket wheel of the Price meter is such that vertical motion, such as that imparted to the meter by pitching or rolling of the boat, will decelerate the bucket wheel, causing low readings. Similarly, excessive swinging of the boat, as may be caused by stiff breezes when no stern anchor is used, will lead to erroneously high readings. Consequently the Price meter can be used from a small boat only under good weather conditions. The salinometer is not bothered by the problems mentioned above. The control unit and transducer themselves are not affected by motion and can be used in rough weather as long as due care is taken to prevent damage to the transducer by its hitting the bottom or the side of the boat.

Data:

Except as noted earlier in the case of the bathymetry, the data shown are believed to be representative of conditions in the lagoon during the months of September and October, 1963. It should be noted, however, that neither the current meter nor the salinometer have been calibrated by the user. Rough checks on the current meter were made by noting the length of time required for surface detritus to drift the length of the boat and calculating velocity from this; the velocities so obtained did not differ significantly from those registered by the current meter. One field check on the salinometer was made with hydrometers and a mercury thermometer, on surface water sampled with a Frautschy bottle held horizontally just under the surface. A measurement was made with the salinometer immediately following the taking of the sample. The salinometer recorded 29.9 o/oo and 13.3 C while the hydrometer indicated 30.1 o/oo and 13.6 C, which values are within the tolerances stated by the manufacturer of the instrument.

A second method of checking salinity and temperature was available. The Shellfish Sanitation Laboratory has a Foxboro recording salinometer and a Tag recording thermometer which continuously record the salinity and temperature of the sea water furnished to several salt-water aquaria. The intake of this system lies on the floor of the mouth of the lagoon, about a hundred feet north of station D. During the morning of 11 October, when station D was occupied, the laboratory instruments recorded temperatures and salinities in the ranges 14.5-15.0 C and 29.5-29.7 o/oo respectively, while, as shown in Table 3, the portable instrument recorded average temperatures and salinities of 13.5 C and 29.6 o/oo. The salinities compare very well; the temperatures less so, perhaps because of the heating effect of the pumping system which is located in a room usually considerably warmer than the sea water (the thermograph probe is located on the outlet side of the pump several feet from the pump itself). Bearing the above in mind, there does not seem to be any reason to doubt that the values shown in the appended tables are not reasonably accurate.

Future Work:

Several things have been mentioned in this report which deserve further observation. First among these is the bathymetry. In order to resolve the doubts expressed above about the data, it would be desirable to have a portable tide gage somewhere in the lagoon, perhaps in the vicinity of the power towers in the southern end. Data from such an instrument covering a month or two would settle the question of time lag between the lagoon and Wauna. If a tide gage were not available, a tide staff might be used, fixed to the power line towers or a bridge pier at the mouth of the lagoon. If a staff were used, regular observations several times a day would be desirable.

Second, the current data should be completed so that a set of observations based on the same tidal difference for both ebb and flood tides would be available. A considerable amount of the observation recorded herein was based on a tidal range of about ten feet. If a full set of data based on this range were available, any generalizations about the current patterns in the lagoon would be on a firmer base. It would be desirable to have several more stations for current data to supplement those present in the southern end of the lagoon.

Third, there should be a full year's data on surface and mid-depth or bottom salinity and temperature, for the data presented here show nothing of the effect of precipitation and runoff on the lagoon. That such effect exists is shown by the fact that at the time of this writing (late November, 1963) the Foxboro recording salinometer mentioned above is showing salinities as low as 20 o/oo on the very low tides which occur at this time of year. But it has been raining almost continually during the month of November, and very little rain was recorded during the time the data were taken in September and October. Data on salinity and temperature taken at several points in both Burley Lagoon and Henderson Bay would show any stratification present, the extent of mixing at various locations, and the duration of such effects through the seasons of the year.

Finally, some investigations should be carried out which time and facilities did not permit at this time. Among these are a survey of the water chemistry of the lagoon, including dissolved oxygen, nitrate and phosphate; and an examination of the mixing process occurring as the water from Burley Lagoon spills into Henderson Bay. It is suspected that mixing is quite complete in this area, as the flow is noticeably turbulent, but more concrete evidence of this would be desirable.

CONCLUSIONS

Instrumentation:

For the work carried out, the instruments used proved serviceable and easy to use, and the use of these instruments is recommended for work of the sort described in this report. The limitations of the Price current meter, i.e. sensitivity to vertical motion and lack of a direction sensor which would allow its use in murky water, are not critical so long as one chooses the times and conditions of observation with these limitations in mind. The inconveniences involved in operation of meters of the Ekman type from a small boat, and the relatively great cost involved in such remote-reading meters as the Kelvin-Hughes and Hytech instruments are also factors which favor the Price meter for work such as that described.

The RS 5-2 salinometer is well suited for this work. Its simplicity and the rapidity with which readings may be made with it especially commend its use. The model described in this report is not limited to the depths at which it was used. The instrument is available with up to 300 feet of cable, which means the RS 5-2 can be used in almost any estuarine situation where high precision is not required. The manufacturer of this instrument now has available a Model RS-6 which measures conductivity, temperature, and depth; and a Model RS-7A, which is a high accuracy instrument apparently similar to the RS 5-2, but neither of these have been investigated by the author.

Burley Lagoon:

During the course of the study, the conditions in Burley Lagoon were much as would be expected. The lagoon flushes almost completely on each tidal cycle, and the quality of the water in the lagoon is dependent almost exclusively on the conditions in Henderson Bay, which is the source for the salt water in Burley Lagoon. The effect of runoff from Burley and Purdy Creeks was nonobservable, so such fresh water as was contributed by these streams must have been well mixed with the salt water not far from the mouths of the streams. These conditions prevail during the summer and early fall, when evaporation dominates over precipitation and runoff; the effects prevailing during those seasons when precipitation and runoff dominate will of course be quite different.

Finally, as suggested earlier in this report, further work is needed. Until such future work on currents and runoff as well as water chemistry is undertaken, the knowledge of the hydrography of Burley Lagoon cannot be considered complete.

ACKNOWLEDGEMENTS

The author wishes to express his gratitude to the members of the Northwest Shellfish Sanitation Research Laboratory for their continued interest during the course of this project, and for their many questions and suggestions. Thanks are especially due to Leroy C. Myers for his assistance in the gathering of data and for his instruction in small-boat handling.

Thanks are due also to Dr. Clifford A. Barnes, of the Department of Oceanography of the University of Washington, for reading the final draft and offering helpful comments about the presentation of the data.

COMMENTS BY PARTICIPANTS

Mr. Sam Reed commented on what emphasis could be placed on the study. How would this or similar studies be utilized in the shellfish certification program: What information could the various states use to implement their individual programs in shellfish sanitation? Mr. Beck replied that the initial program would be collated with other studies on Burley Lagoon such as the bacteriological and sanitary surveys performed as cooperative effort between Washington State Department of Health and the Shellfish Laboratory. Further investigations in oceanography would be necessary before the entire relationship could be completed.

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Table 1. Soundings in Burley Lagoon

Sounding No.	Depth re mhhw* feet	Sounding No.	Depth re. mhhw* feet	Sounding No.	Depth re. mhhw* feet
1	9	30	14	59	14
2	10	31	15	60	13
3	9	32	15	61	14
4	9	33	16	62	12
5	10	34	15	63	10
6	12	35	15	64	12
7	10	36	14	65	14
8	10	37	16	66	15
9	13	38	11	67	23
10	12	39	21	68	16
11	12	40	15	69	17
12	11	41	12	70	20
13	12	42	12	71	23
14	13	43	14	72	26
15	9	44	15	73	23
16	11	45	16	74	27
17	12	46	18	75	24
18	11	47	13	76	21
19	12	48	15	77	22
20	11	49	17	78	13
21	11	50	21	79	14
22	13	51	31	80	15
23	13	52	25	81	19
24	12	53	19	82	21
25	13	54	15	83	19
26	13	55	18	84	18
27	14	56	18	85	15
28	14	57	18	86	19
29	14	58	16	87	19

*mhhw = mean higher high water

Table 2. Tidal data for Wauna, Washington

High water	+0h 20m	+ 1.8 feet	add to Seattle prediction
Low water	+ 0h 36m	+ 0.0 feet	" " " "

Mean higher water	13.1 feet
Mean high water	12.2
Mean low water	02.8
Mean lower low water	00.0
Mean range	09.4
Diurnal range	13.1
Mean tide level	07.5

Table 3. Average surface and mid-depth temperatures and salinities for Burley Lagoon and Henderson Bay

Station	Surface		Mid-depth	
	Av. S o/oo	Av. T° C	Av. S o/oo	Av. T° C
A	29.4	13.7	29.7	14.1
B	29.5	14.0	29.5	13.8
C	29.5	14.4	29.6	13.5
D	29.6	13.5	-	-
E	29.8	13.5	30.0	13.3
F	29.2	13.7	29.7	13.4
G	29.5	13.2	29.9	13.0
H	29.3	13.3	29.6	13.0
Mean	29.5	13.7	29.7	13.4

Table 4. Gage height and stream discharge for Burley and Purdy Creeks September-October, 1963

	Burley Creek				Purdy Creek			
	September		October		September		October	
	Gage Height ft.	Discharge Acre ft. /day	Gage Height ft.	Discharge Acre-ft. /day	Gage Height ft.	Discharge Acre-ft. /day	Gage Height ft.	Discharge Acre-ft. /day
Max.	1.18	23.0	1.90	67.0	0.48	03.5	0.68	12.5
Min.	1.00	11.5	1.06	15.5	0.36	01.0	0.38	01.5
Avg.	1.05	15.0	1.21	25.0	0.39	01.5	0.44	02.5

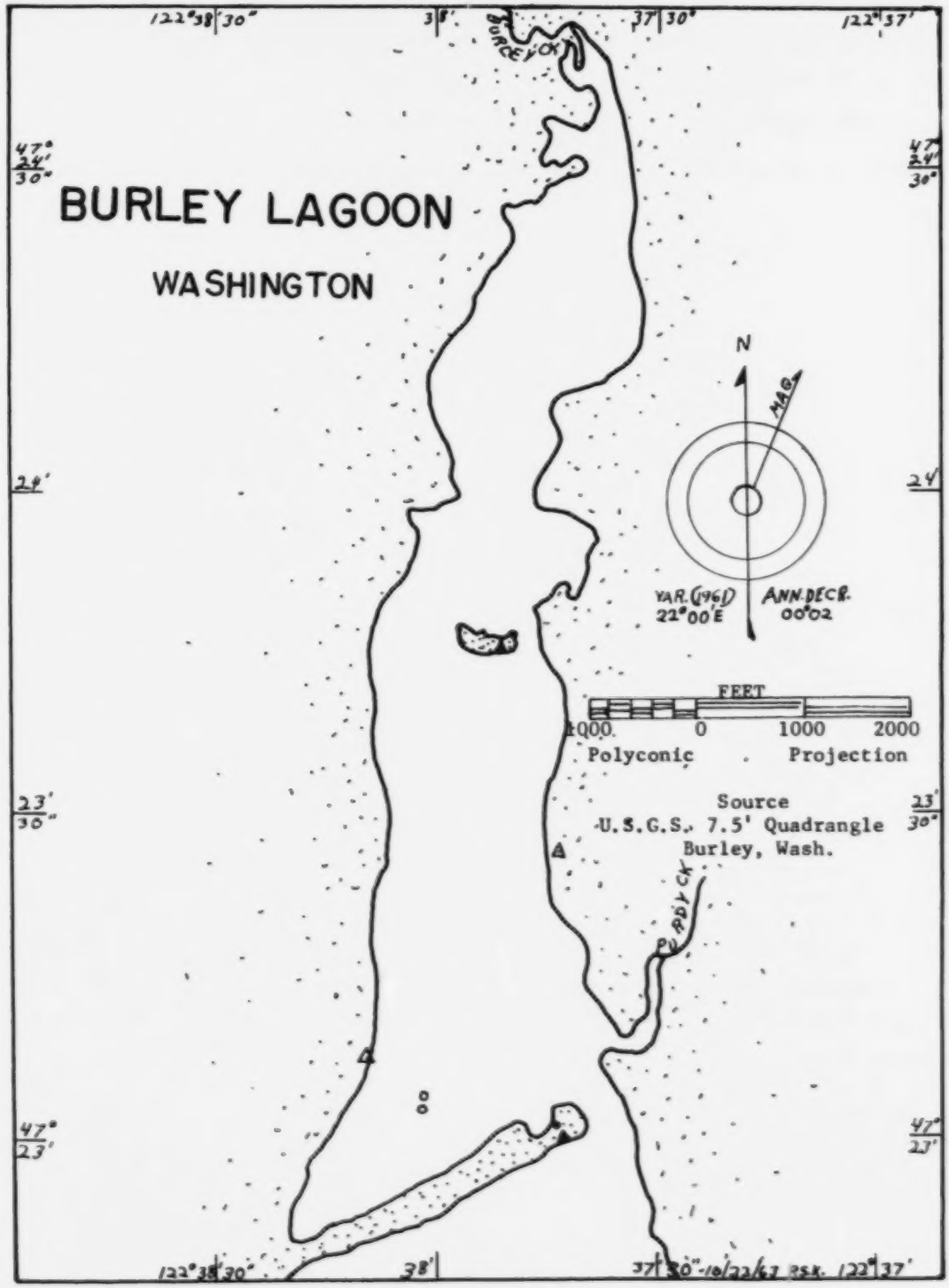

Fig. 1 Base Map of Burley Lagoon, on Henderson Bay at the Head of Carr Inlet, Southern Puget Sound

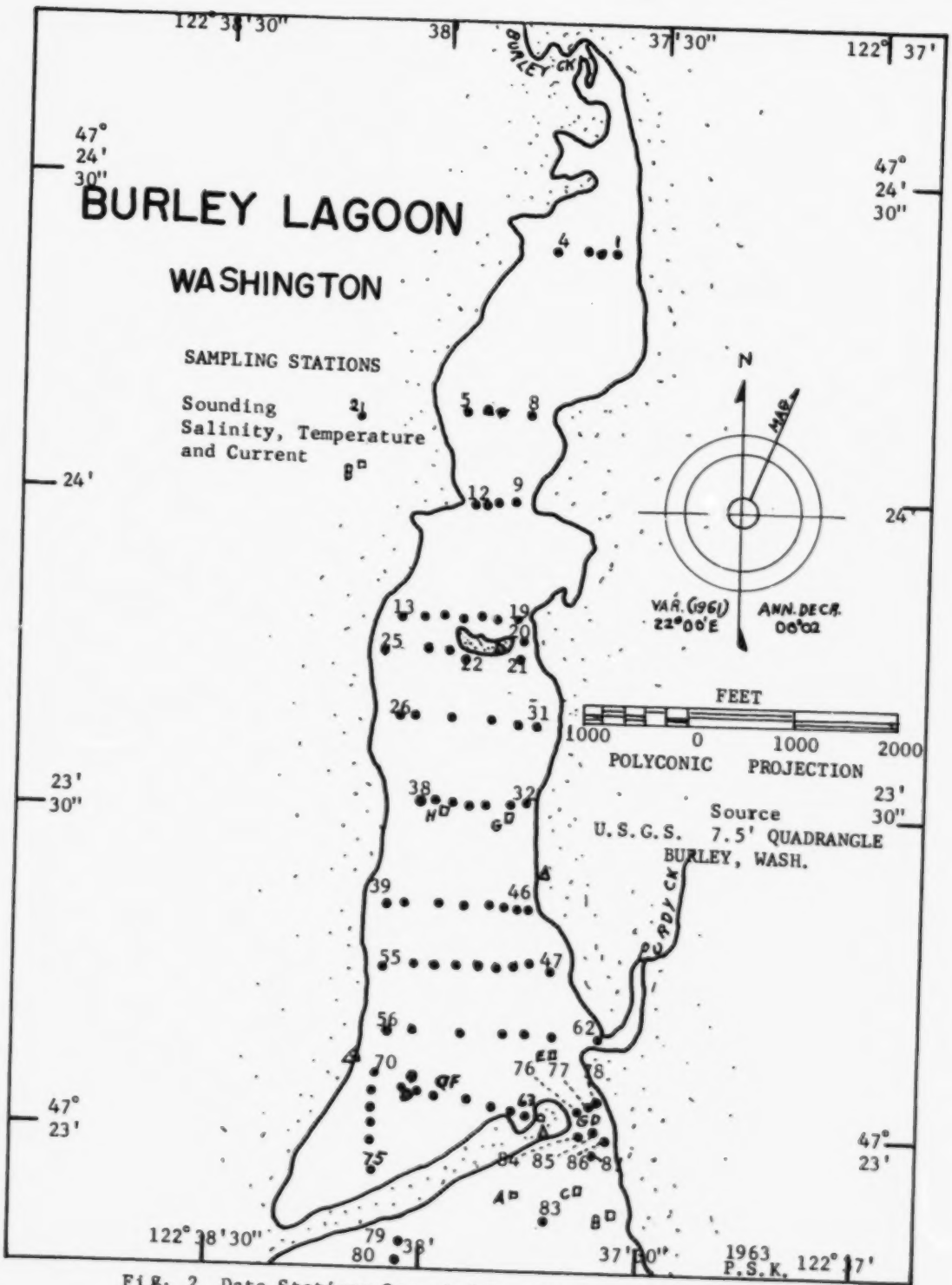

Fig. 2 Data Stations Occupied For Bathymetry, Temperature, Salinity, and Currents on Burley Lagoon and Henderson Bay

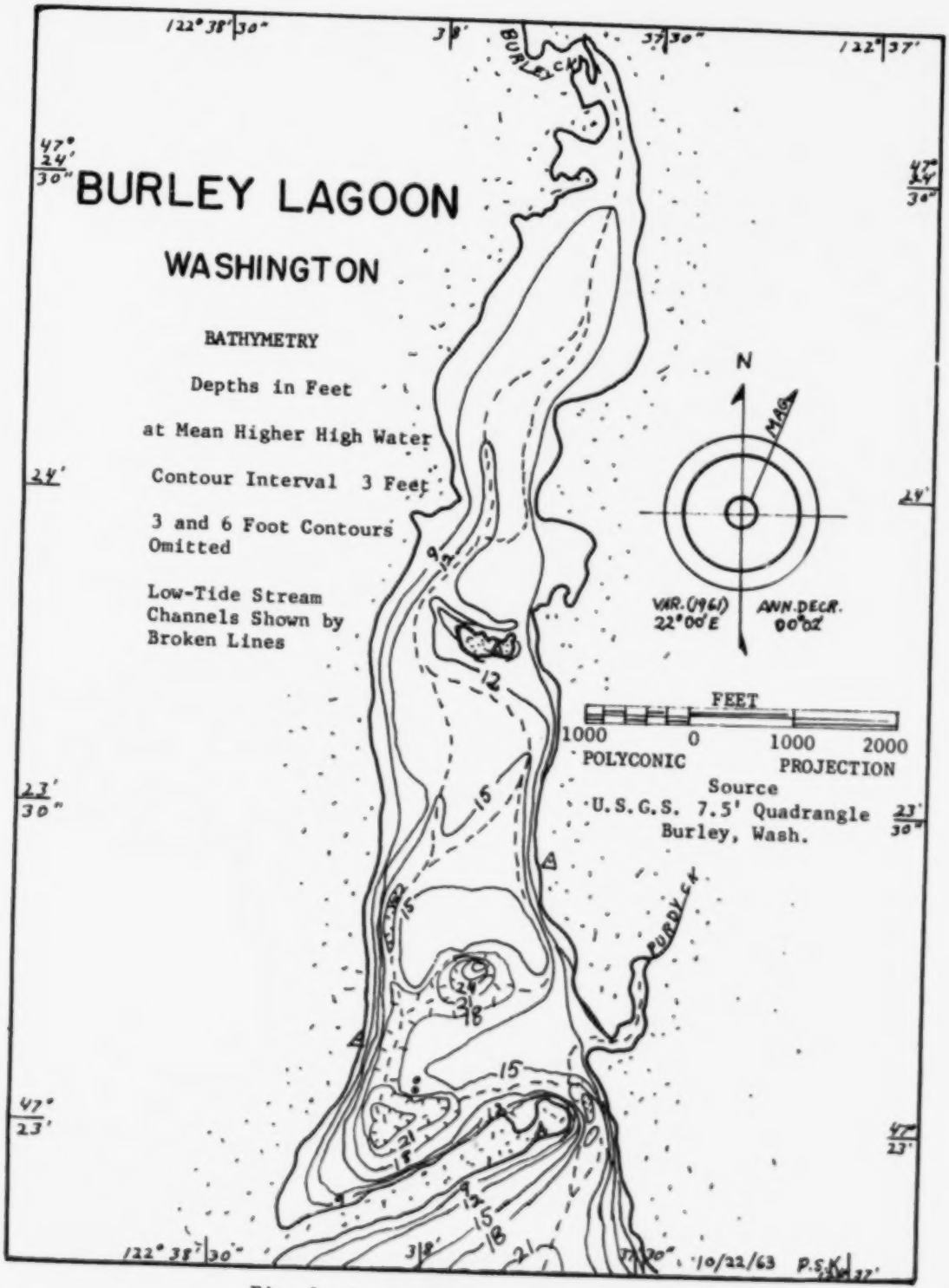

Fig. 3 Contour Chart of Burley Lagoon

Fig. 4
Price Current Meter, Salt-Water Modification, Made by W. and L. E. Gurley. Battery and Electric Counter Shown at Right, Separated from Meter by 30 feet of Cable.

Fig. 5
View from Bow of 16-foot Outboard Motorboat, Showing Detail of Boom and Cable Clamp for Current Meter.

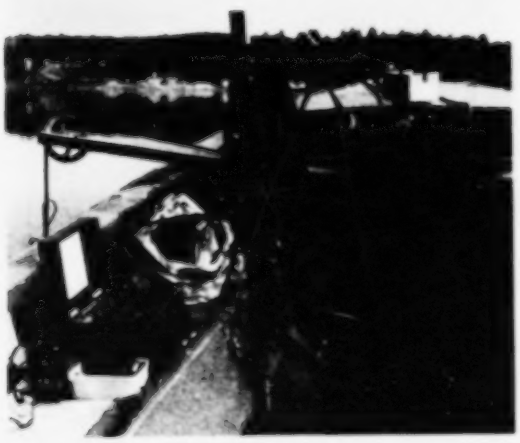

Fig. 6
View from Stern of Boat, Showing General Layout of Equipment. Pole in Center Holds Cables for Current Meter and Salinometer. Meter Counter and Salinometer Control are at Left. Box to Right of Steering Wheel Holds 4-inch Boat Compass.

Fig. 9
Industrial Instruments Model RS5-2 Portable Salinometer. Transducer is at Right, Separated from Control by 100 feet of Cable.

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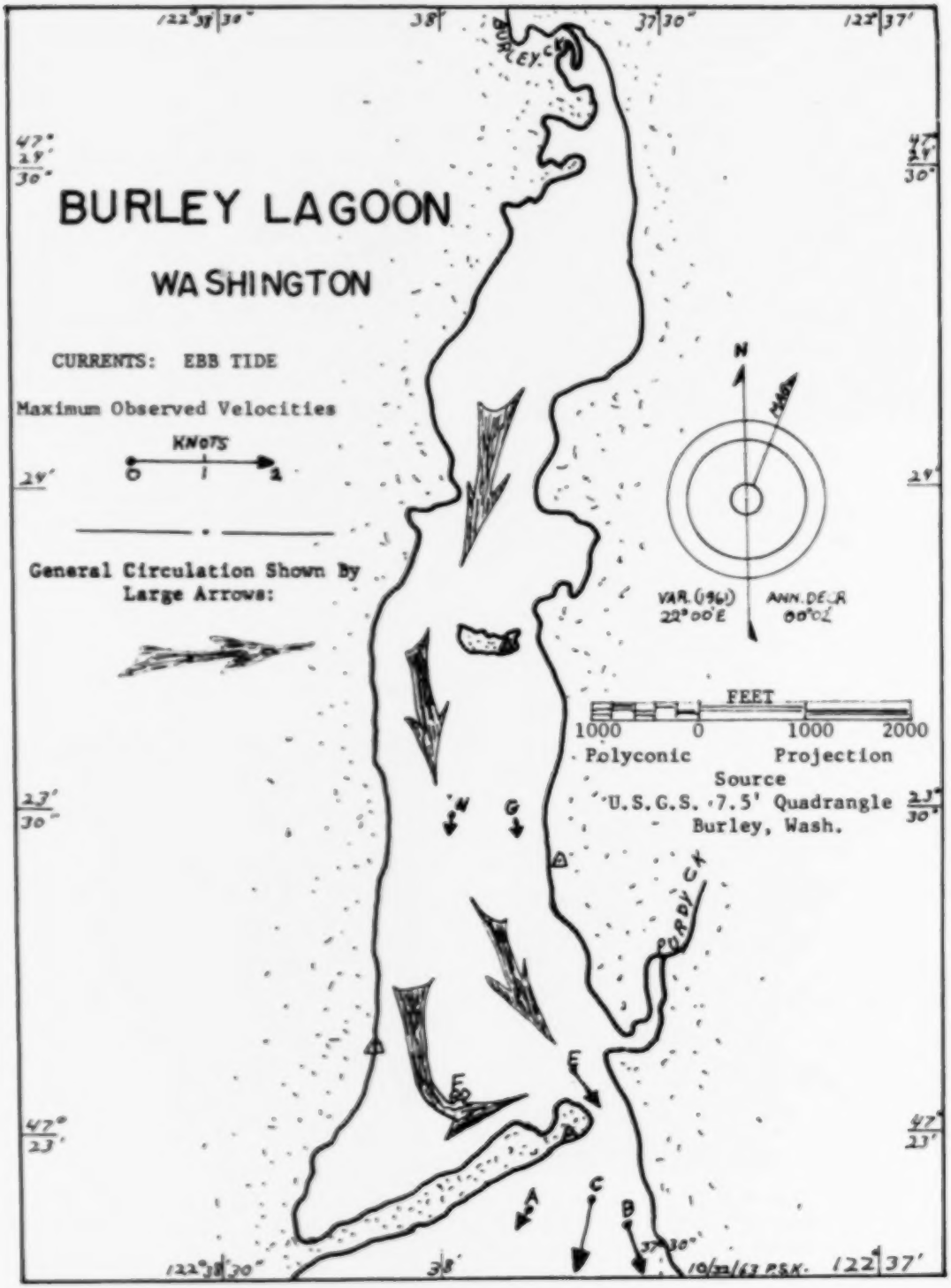

Fig. 7 Ebb Tide Current Patterns in Burley Lagoon

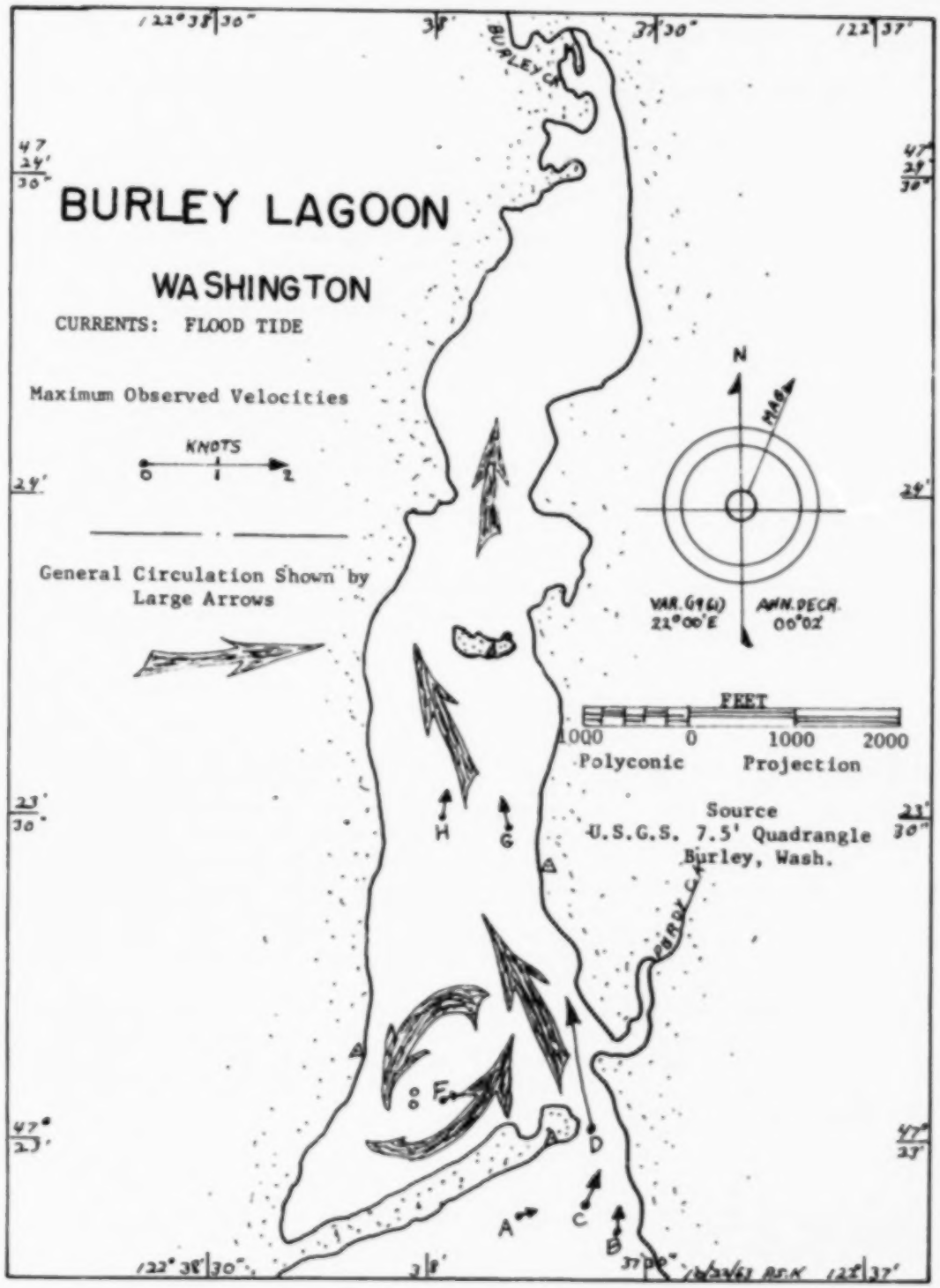

Fig. 8 Flood Tide Current Patterns in Burley Lagoon

Bacteriological Study of Stored Pacific Oyster Shellstock

J. C. Hoff, W. J. Beck and T. H. Ericksen

INTRODUCTION

Several indices used in other areas of sanitary bacteriology have been used to determine the microbiological quality of fresh, packed oysters in commercial channels. The changes which occur in these indices during storage of shucked Pacific oysters have been extensively studied in this laboratory. However, the effects of storage of shellstock on these indices have not been investigated.

In commercial operations, dry storage of oyster shellstock for two or more days at temperatures of 10 C or above is not unusual. Under these conditions, shucked oysters show rapid changes in bacterial populations and deteriorate rapidly. Therefore, it is of interest to determine whether or not similar changes occur in the living oyster stored under these conditions.

This study was undertaken to investigate the behavior of several bacterial indices during dry storage of Pacific oyster shellstock. The study was initiated in September 1963 and continued through August 1964.

MATERIALS AND METHODS

Oysters. The oysters used in this study were obtained through commercial sources in Washington and Oregon. Each lot consisted of approximately 370 oysters, 10-15cm long. The oysters were scraped to remove barnacles and other fouling organisms and washed in fresh water to remove mud on arrival at the laboratory. Ten lots were collected locally directly from the beds by laboratory personnel. These lots were placed in storage within four hours after harvesting. Two lots, collected in Oregon, had been collected some time previous to arrival of laboratory personnel. The time elapsed between harvest and storage on these two lots is uncertain.

Storage. Approximately 120 oysters were stored at 10 C, 20 C and 27.5 C respectively. Storage facilities consisted of refrigerators equipped with refrigeration or heating units and thermoregulators capable of controlling temperatures to ±0.5 C. The oysters were placed one layer deep on racks in these units.

Sampling. Samples consisted of 10 tightly closed oysters. One sample tested on arrival at the laboratory constituted the 0 hr. sample for all three temperatures. Sampling schedules following the initial sample were as follows:

10 C - 1,2,4,7,10,15,20, and 25 days.

20 C - 1/2, 1,1-1/2,2,3,4,5,6, and 7 days.

27.5 C - 1/2,1,1-1/2,2,3, and 4 days.

"Gaping" oysters were also examined if more than five were found. Excess "gapers" were discarded at each sampling time.

Examination. The bacteriological indices determined on each sample consisted of five tube coliform and fecal coliform MPN's, and standard plate counts at 35 C performed according to recommended procedures (1962). The pH was determined electrometrically on a portion of the blended sample. At selected intervals IMViC tests on cultures isolated from EC positive tubes were performed to determine whether or not a differential dieoff of Escherichia coli occurred during storage.

Storage Schedule. Storage experiments were planned so that several lots were examined in each season. In this way, possible effects of seasonal variations in the oysters and their bacterial flora on the behavior of the bacterial populations during storage could be evaluated.

RESULTS

The chronology of the storage experiments, sources, and initial groupings for data analysis are shown in Table 1. Geometric mean values for each bacterial index in each seasonal group were calculated and the results were plotted. The changes shown by the indices in all four groups were similar. Therefore, geometric means derived from all 12 lots were calculated and plotted. In a few instances, due to laboratory accidents or indeterminate results, data were not available for a particular sampling time. In some lots, storage was terminated earlier than in others because no tightly closed oysters remained. In these instances the results are based on fewer than 12 lots.

The results of storage of the 12 lots at 10 C are shown in Fig. 1. The fecal coliform MPN remained quite stable but showed a slight overall decline during the 20-day storage period. Coliform MPN's increased about twofold during the first 2 days after which they remained relatively stable. Plate counts increased about fourfold during the first day and continued to increase at a slower rate during the remainder of the storage period.

At 20 C (Fig. 2), the storage time was considerably shortened because the animals were usually all "gapers" by the seventh day. Fecal coliform MPN's increased about twofold during the first two days and remained stable at that level for the remaining period. Coliform MPN's increased approximately ninefold during the first two days then remained relatively stable. Plate counts increased twenty-eightfold during the first day of storage, then continued to increase at a slower rate.

The results of storage at 27.5 C are shown in Fig. 3. Oysters survived for only 3 days at this temperature. Numbers of all 3 indices increased rapidly during the first day and continued to increase at a rapid rate.

The results of IMViC tests on isolates from EC positive tubes are shown in Table 2. At 10 C, no change in percent E. coli found is evident. At 20 C and 27.5 C E. coli percentages appeared to drop slightly as storage time progressed. However, the change if any was slight.

The results of pH determination are shown in Table 3. It is obvious that pH in the live Pacific oyster was not affected by changes in bacterial populations within the animal.

"Gapers" were not always found in sufficient numbers to constitute a sample at each sampling interval in each lot. When three or more comparative samples of closed and "gaping" oysters were examined, geometric means and ratios of "gaper" populations to closed oyster populations were calculated. The results are shown in Table 4. The 35 C plate counts showed the greatest differences and were always as high or higher when the "gapers" were compared with closed oysters.

DISCUSSION

Previous studies have shown that the fecal coliform group enumerated by the EC test was influenced least by duration or condition of storage in shucked Pacific oysters, (Presnell, 1962; Hoff, Beck, and Presnell, 1964) and in the Eastern oyster (Presnell and Kelly, 1961).

The data presented in this study indicated that the above statement also applies to dry stored Pacific oyster shellstock. Although fecal coliform MPN's did increase at 27.5 C little change occurred at 20 C and 10 C. Rapid death (gaping) and rapid increases in plate count, coliform and fecal coliform populations in shellstock stored at 27.5 C indicate that shellstock should not be held in areas where temperatures in this range persist.

Evidence for the biological soundness of the use of the fecal coli form group as an indicator of fecal pollution by man and other warm blooded animals has been given previously (Kelly, 1960; Presnell, 1961; Kelly et al, 1962; Beck, Presnell, and Hoff, 1963; Kabler, Clark and Geldreich, 1964). Because of this and because of the stability of this index during storage indicated above, it appears that the EC group would be of value as an indicator of the sanitary quality of Pacific oyster shellstock during the interim between harvesting and shucking.

The 35 C plate count showed the most uniform differential response to storage time and temperature. At 10 C the time required for a ten-fold increase to occur was five days while at 20 C a tenfold increase occurred in one-half day. It is apparent that either low shellstock storage temperatures or prompt shucking of harvested shellstock would be conducive to lower initial plate count populations in the product and thus to longer shelf life.

The response of the coliform group to storage time and temperature was less consistent and uniform than that of the 35 C plate count and less stable than that of the fecal coliform group. These factors, in combination with its doubtful validity as an indicator of sanitary quality indicate that the coliform index would be less valuable as an indicator of shellstock bacteriological quality.

Despite large increases in populations which occurred at the higher storage temperatures, the pH showed little change. Therefore, it appears that this criterion would give no information as to bacteriological quality of Pacific oyster shellstock.

COMMENTS BY PARTICIPANTS

Dr. Liston discussed the use of 20 and 27.5 C dry storage. Mr. Kelly commented on the lack of change in fecal coliform make-up at the three storage temperatures. Dr. Hoff made further comments which included the difference in plate counts and the possibility of differential control of microorganisms by the animal.

Mr. Girard asked about difference between liquors and meats. Mr. Kelly commented on other studies on the Gulf Coast where some multiplication evidently takes place in the animal and liquor. Drs. Dollar and Hoff discussed the reasons for the loss of liquor by some oysters. Again the relationship of pathogenic organisms to the indicator organisms were discussed. Mr. Kelly and Dr. Bartsch commented on the difficulties of obtaining samples with known pathogenic organisms.

Drs. Sparks and Quayle commented on length of time shellfish can be held in dry storage under various conditions.

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Table 1. Chronology of Pacific oyster shellstock storage study

Lot	Date Stored	Source	Grouping for Initial Analysis
2	9-30-63	Washington)	
3	10-14-63	Washington)	3 lots - Fall
4	10-28-63	Washington)	
5	1- 6-64	Washington)	
6	1-27-64	Oregon)	4 lots - Winter
7	2-10-64	Oregon)	
8	2-24-64	Washington)	
9	5-11-64	Washington)	
10	5-18-64	Washington)	3 lots - Spring
11	6- 8-64	Washington)	
12	7-20-64	Washington)	2 lots - Summer
13	7-27-64	Washington)	

Table 2. IMViC analysis of E C positive cultures isolated during storage of Pacific oyster shellstock (12 lots)

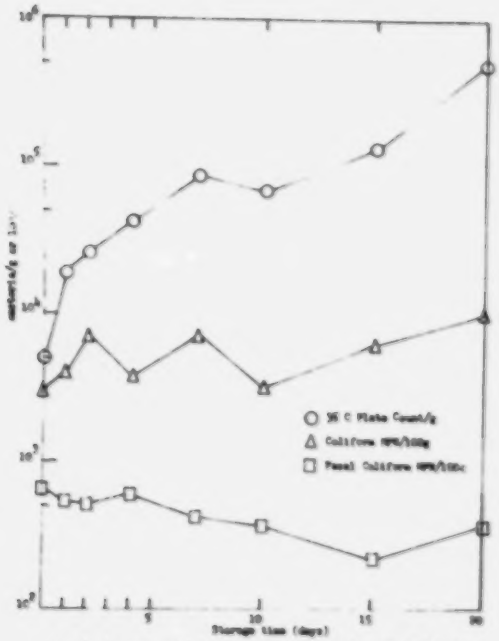
Storage time (days)	10 C		20 C		27.5 C	
	Total No. Isolates	% E.coli	Total No. Isolates	% E.coli	Total No. Isolates	% E.coli
0	88	97	88	97	88	97
1/2-1	-	-	114	96	197	95
1-2	56	91	-	-	-	-
1 1/2-2	-	-	121	89	198	92
3-4	-	-	144	88	107	84
4-7	107	91	-	-		
5-6	-	-	125	87		
10	74	95				
15	68	100				
20	76	99				

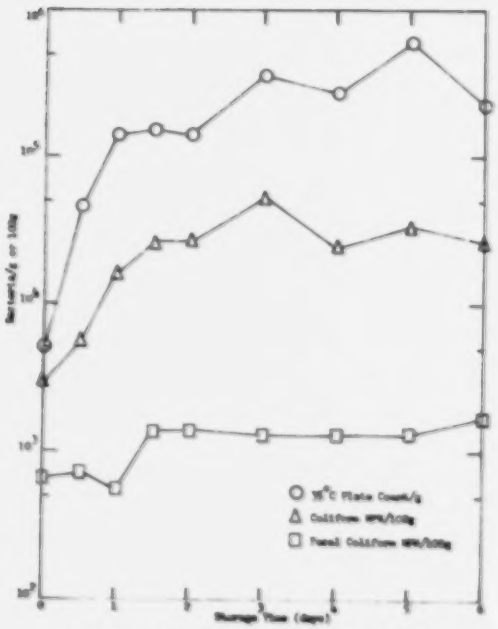
Table 3. Effect of storage time and temperature on pH of Pacific oyster shellstock

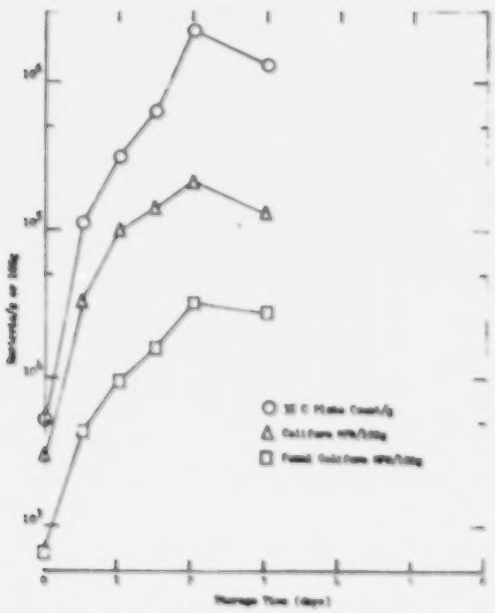
Storage Time (days)	10 C	20 C	27.5 C
0	6.5	6.5	6.5
1/2		6.4	6.4
1	6.4	6.4	6.4
1 1/2		6.4	6.4
2	6.4	6.5	6.3
3		6.5	6.1
4	6.5	6.5	
5		6.4	
6		6.3	
7	6.5		
10	6.5		
15	6.5		
20	6.5		

Table 4. Comparison of bacterial populations in gaping and closed Pacific oysters

Time (days)	Ratio: Bacteria in "gapers" / Bacteria in closed oysters — 27.5 C: Coliform MPN/100g	27.5 C: Fecal Coliform MPN/100g	27.5 C: 35 C Plate Count/g	20 C: Coliform MPN/100g	20 C: Fecal Coliform MPN/100g	20 C: 35 C Plate Count/g	10 C: Coliform MPN/100g	10 C: Fecal Coliform MPN/100g	10 C: 35 C Plate Count/g
1 1/2	1.1	3.5	1.2						
2	3.0	2.3	1.9						
3	3.9	3.4	13.1	1.1	1.4	2.6			
4				0.3	0.7	2.3			
5				0.7	1.1	1.1			
6				1.2	0.4	10.0			
10							12.5	2.0	16.1
15							1.3	0.8	9.3
20							0.9	0.8	2.0







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Storage Studies on Manila Clams (Tapes japonica) and Native Littleneck Clams (Protothaca staminea) shellstock

T. H. Ericksen, J. C. Hoff, and W. J. Beck

INTRODUCTION

This study was initiated to determine the effects of a range of storage temperatures on the bacterial quality of clam shellstock as indicated by the 35 C plate count, coliform MPN and fecal coliform MPN. Recommendations by previous Shellfish Sanitation Planning Conferences were made in order that aid in evaluation of present marketing practices of Manila (Tapes japonica) and Native Littleneck (Protothaca staminea) clams could be made. The study consisted of eleven lots of each species of clams collected at seasonal intervals over a period of one year.

MATERIALS AND METHODS

The clam shellstock was collected at various sites in the State of Washington through the cooperation of the Washington State Department of Health. The first 2 lots were collected by commercial harvesters and the remaining lots by laboratory personnel of the Northwest Shellfish Sanitation Laboratory. Transport of samples to the laboratory and bacteriological examination were carried out as described in APHA Recommended Procedures for the Bacteriological Examination of Sea Water and Shellfish (Third Edition, 1962).

Each lot of clam shellstock consisted of 360 individuals. Each lot was divided into three equal groups which were stored at 10 C, 20 C and 27.5 C respectively. Both species were stored and examined simultaneously. Perforated plastic bags were used to store clams to simulate market storage practices. Modified refrigerators equipped with electronic relays, constant temperature recording devices and the appropriate thermal regulators were used for storage. Each sample for examination consisted of a minimum of five clams and a maximum of ten clams for each species.

Clams stored at 10 C were examined at 0, 1, 2, 4, 7, 10, 15 and 20 days. Clams stored at 20 C and 27.5 C were examined at 0, 1/2, 1, 1-1/2, 2 days and 1 day intervals thereafter until no clams remained closed. The inability to close its shell in response to tactile stimuli was an arbitrary criterion for determining the clam sample to be examined.

Samples were selected at each temperature and prepared and shucked in the prescribed manner. Each sample of shellfish meats and liquor was weighed in a sterile tared beaker. The shellfish sample was placed in a sterile blending jar and blended for twenty seconds, after which a 20 ml aliquot of homogenate was removed. The loss of the 20 ml aliquot was compensated for and a weight of phosphate buffer equal to that of the shellfish homogenate remaining in the blender jar was added. This mixture was blended for an additional eighty seconds.

Bacteriological examination immediately followed the blending procedure. The pH of the sample was determined electrometrically from the 20 ml aliquot. EC positive tubes at selected intervals were submitted to the IMViC test. However, because of the selected intervals the number of EC positive tubes submitted to the IMViC test was limited. These data indicated growth differentials of the various fecal coliform organisms present throughout the storage period.

"Gaper" samples, which were determined by the inability of the clam to close its shell to tactile stimuli, were collected simultaneously with closed shellstock samples at each of the three respective temperatures for both clam species. All "gapers" were removed at each sampling period, therefore the clam shellstock of each species remaining at each temperature was closed shellstock.

Modification of storage temperatures and examination intervals were made after testing the first 2 lots of each species. This accounts for the 27.5 C storage temperature being added on the third lot and thereafter.

RESULTS

The results of storage of Native Littleneck clam shellstock are shown in Figs. 1, 2 and 3 and Manila clam shellstock in Figs. 4, 5 and 6.

The coliform MPN and 35 C plate counts were analyzed by the geometric mean method. Because of the presence of indeterminate numbers (<18), median values were used for fecal coliform MPN's. Comparison of values for seasonal series of lots indicated there were no apparent seasonal variations. Therefore, lots at each storage temperature were grouped by sampling time, clam species and specific bacterial index and the geometric means or medians determined. The values represented in the figures were determined from approximately eleven lots each.

During storage of Native Littleneck clams at 10 C (Fig. 1) only a slight change in the fecal coliform MPN occurred. The change was a slight decrease after ten days of storage. The coliform MPN showed an immediate slight decrease for the first two days, which was followed by a leveling-off period. The 35 C plate counts indicated an overall continuous increase as storage progressed.

Native Littleneck clams stored at 20 C (Fig. 2) showed little change in the fecal coliform MPN. However, slight fluctuation was evident. The coliform MPN changed somewhat erratically, but showed a general decrease. The 35 C plate count indicated a steady increase throughout storage.

The 27.5 C storage of Native Littleneck clams (Fig. 3) showed a slight decrease in the fecal coliform MPN. The coliform MPN indicated a continuous increase as storage progressed. The 35 C plate counts showed a steady sharp increase throughout storage.

Overall, the fecal coliform MPN's remained relatively stable during storage at the three temperatures. The coliform MPN's at 10 C and 20 C showed slight decreases and at 27.5 C indicated an increase. The 35 C plate counts showed steady increases at all three temperatures. However, as the storage temperature increased this bacterial index increased at a faster rate.

The Manila clam shellstock stored at 10 C (Fig. 4) showed little change in the fecal coliform MPN during storage. The coliform MPN's showed a slight increase for seven days followed by a decrease for the remainder of the storage period. The 35 C plate counts indicated an initial lag period of two days followed by a steady increase.

Storage of Manila clams at 20 C (Fig. 5) showed little change in the fecal coliform MPN. The coliform MPN indicated a steady increase throughout storage. The 35 C plate counts showed an initial lag period of one-half day followed by a general increase.

In Manila clams stored at 27.5 C (Fig. 6) the fecal coliform MPN decreased. The coliform MPN indicated a steady increase throughout storage. the 35 C plate counts indicated a steady sharp increase.

Overall in Manila clam shellstock the fecal coliform MPN remained relatively stable. However, a general decrease was observed at 27.5 C. The coliform MPN's indicated general increases as storage progressed at the three temperatures. The 35 C plate counts showed general increases at the three temperatures. However, lag periods were observed at 10 C and 20 C. Again, as in the Native Littleneck clams, the 35 C plate counts increased at a faster rate as the storage temperature increased.

The data in Fig. 7 showed relatively little change in the pH of either species at the three temperatures as storage progressed. The values for the Native Littleneck clams were slightly higher than those of the Manila clams.

Bacteriological examination of "gaper" samples at the three temperatures for the two species of clams indicated predominantly higher bacterial densities than samples of closed clams at the same temperature and storage time. The ratio of geometric means of coliform MPN's, fecal coliform MPN's and 35 C plate counts of "gaping" clams to closed clams are shown in Table 1 for Native Littleneck clams and Table 2 for Manila clams. The data indicated generally higher values for "gapers" than closed clams for both species at the three temperatures. No patterns were apparent. Generally the ratios were higher for the Native Littleneck clams. Therefore a marked difference by species was noted.

Results of 489 IMViC tests indicated the predominant fecal coliform was Escherichia coli. Results of these tests are given in Tables 3 and 4. This data indicated a higher percentage of fecal coliform present as E. coli in Manila clams than in the Native Littleneck clams. The percent of E. coli in tested samples of Manila clams ranged from 87 to 100. In Native Littleneck clam shellstock, the percentage as E. coli of fecal coliform organisms tested ranged from 75 to 88 at 10 C; 79 to 100 at 20 C and 65 to 80 at 27.5 C. At all storage temperatures the E. coli percentage remained relatively stable for both species.

Several samples of both species of clam shellstock from various lots have been stored at -5 C. However, only a limited number of these have been tested. To date the results appear to be similar to those of the zero-hour sample.

DISCUSSION

Data obtained from Manila and Native Littleneck clam shellstock stored at 10 C, 20 C and 27.5 C indicated 35 C plate count increased as storage progressed. The coliform MPN also increased for both species of clams except for the Native Littleneck clams stored at 10 C and 20 C. This increase for both indices appeared to be more rapid in the Manila clams. Generally it was shown that as the storage temperature increased the bacterial density increased at a faster rate as indicated by these two indices.

The data indicated there was little change in the fecal coliform MPN's of both clam species at the three temperatures. However, a decrease was observed in Manila clams stored at 27.5 C.

This data agrees with the previous work on shucked Pacific oysters (Presnell, 1962; Hoff, Beck, and Presnell, 1964) and in the Eastern oyster (Presnell and Kelly, 1961). The coliform MPN and 35 C plate count were related to temperature and condition of storage whereas the fecal coliform MPN exhibited no such relationship. Previous studies have shown that the fecal coliform group may be used as an indicator of fecal pollution by warm-blooded animals (Kelly, 1960; Presnell, 1961; Kelly et al, 1962; Beck, Presnell and Hoff, 1963; Kabler, Clark and Geldreich, 1964). Therefore because the fecal coliform MPN of Manila and Native Littleneck clam shellstock did not appear to be affected by duration or condition of storage, this index appeared to be a valued indicator of the sanitary quality of dry stored Manila and Native Littleneck clam shellstock.

It was also shown that each individual index for each respective storage temperature exhibited similar growth patterns for both species of clams. The general coliform MPN, fecal coliform MPN and 35 C plate count changes exhibited by both clam species were similar to the changes exhibited by the same indices for the Pacific oyster shellstock storage studies.

The pH data indicated relatively no appreciable change during storage at the three temperatures for both species of clam.

Data obtained from IMViC tests of selected positive EC tubes indicated the predominant fecal coliform organism present was E. coli. From this data no differential growth patterns of different fecal coliforms could be discerned during storage at the three temperatures. However, there was a higher percentage of fecal coliforms present as E. coli in Manila clams. The differences between Manila and Native Littleneck clam results may be explained by the fact that earlier studies indicated Manila clams possibly concentrate these organisms to a greater degree. Also, the Manila and Native Littleneck clams were collected from different areas and therefore may have been subject to dissimilar environments. The changes exhibited by the coliform MPN and 35 C plate counts for both species of clams during storage also may have influenced this situation.

The data from the "gaper" study indicated that the inclusion of "gaping" clam shellstock possibly would increase the bacterial density of samples. The ratios for the Native Littleneck clams tended to be greater than those of Manila clams. Therefore, a species difference was apparent. It was also observed that the Native Littleneck clams remained closed longer at all temperatures than the Manila clams.

From this study it may be concluded that of the three storage temperatures, 10 C would be the better dry storage temperature for Manila and Native Littleneck clam shellstock. The coliform MPN and 35 C plate count seemed to be related to duration and condition of storage. The fecal coliform did not exhibit this relationship.

COMMENTS BY PARTICIPANTS

Mr. Kelly pointed out the differences between fecal coliform results as compared with plate counts and coliform results at 27.5 C.

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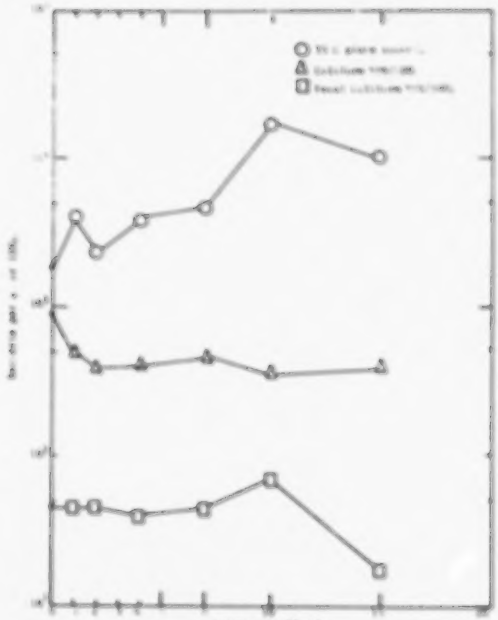

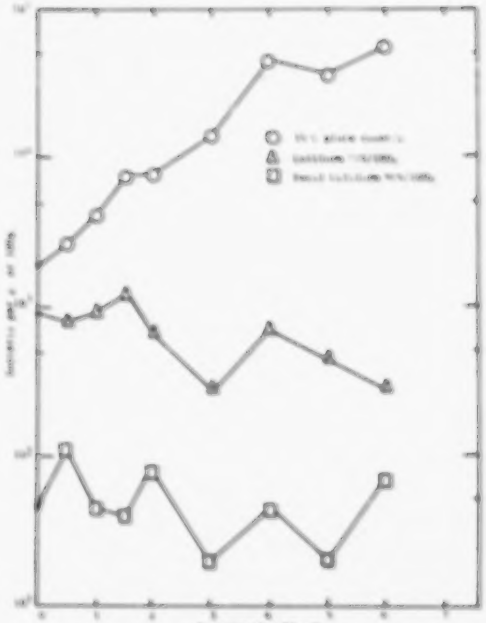

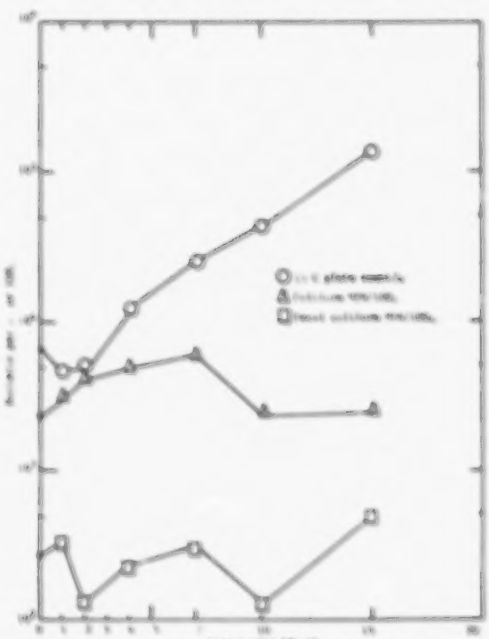

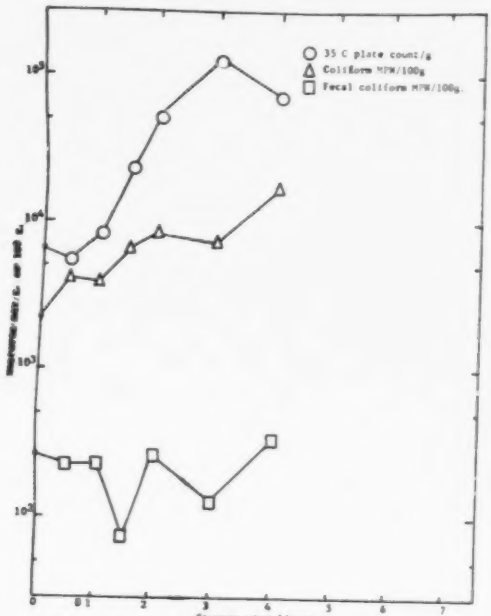

Fig. 5. Changes in coliform MPN, fecal coliform MPN, and 35 C plate count in Manila clam shellstock stored at 20 C (11 lots)

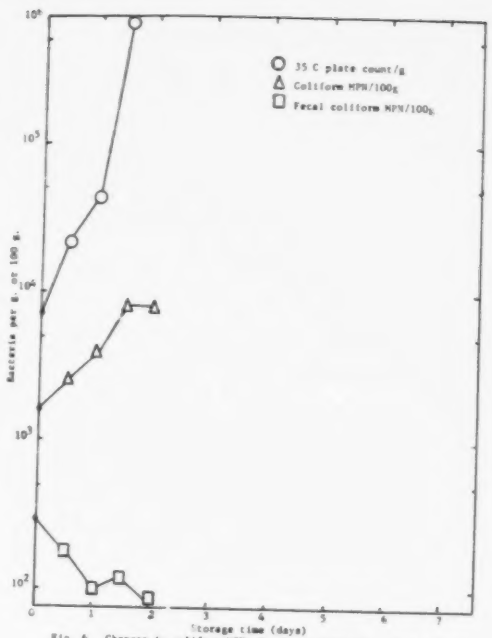

Fig. 6. Changes in coliform MPN, fecal coliform MPN, and 35 C plate count in Manila clam shellstock stored at 27.5 C (11 lots)

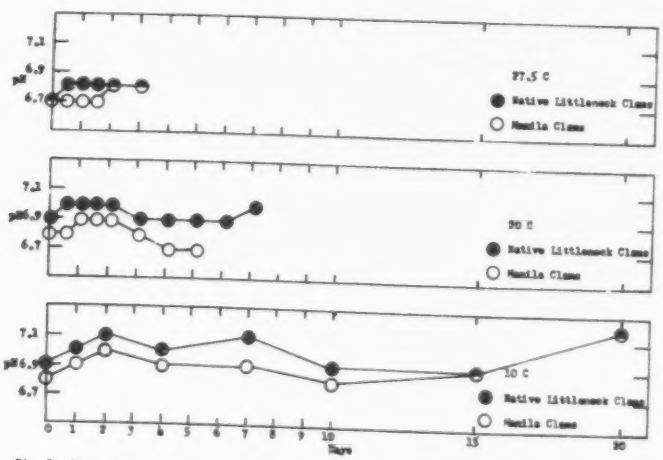

Fig. 7. Changes in pH of Eleven Lots of Manila and Native Littleneck Clams Stored at 10, 20, and 27.5 C

Table 1. Comparison of bacterial populations in "gaping" Native Littleneck clams with those in closed Native Littleneck clams

Storage Time (days)	Ratio Bacteria in "gapers" * / Bacteria in closed clams *								
	27.5 C			20 C			10 C		
	Coliform MPN	Fecal Coliform MPN	35 C Plate Count	Coliform MPN	Fecal Coliform MPN	35 C Plate Count	Coliform MPN	Fecal Coliform MPN	35 C Plate Count
1/2	1.36	1.13	3.57						
1	1.34	0.79	1.67						
1 1/2	14.29	52.45	18.57	4.12	14.29	7.06			
2	2.0	1.93	3.75						
3	1.75	9.33	10.48	5.93	7.81	7.78			
4				5.13	0.67	10.00			
5				1.58	3.05	6.67			
6				6.25	0.63	4.86			
7							4.89	1.47	2.95
10							1.35	1.16	3.64
15							2.57	2.00	5.71
20							0.27	0.92	21.21

*Geometric means

Table 2. Comparison of bacterial populations in "gaping" Manila clams with those in closed Manila clams

Storage (days)	Ratio: Bacteria in "gapers" * / Bacteria in closed clams *								
	27.5 C			20 C			10 C		
	Coliform MPN	Fecal Coliform MPN	35 C Plate Count	Coliform MPN	Fecal Coliform MPN	35 C Plate Count	Coliform MPN	Fecal Coliform MPN	35 C Plate Count
1/2									
1									
1 1/2	1.58	1.35	2.89						
2	0.86	0.60	1.38	0.76	0.55	10.38			
3	0.46	2.43	1.25	2.29	2.24	1.74			
4				1.41	0.85	0.91			
5				1.33	1.43	1.89			
7									
10									
15							1.1	2.0	1.42

*Geometric means

Table 3. IMViC analysis of EC positive cultures isolated during storage of Native Littleneck clam shellstock

Storage time (days)	10 C		20 C		27.5 C	
	Total No. Isolates	% E.coli	Total No. Isolates	E.coli	Total No. Isolates	% E.coli
0	28	79	28	79	28	79
1/2	-	-	21	100	20	65
1	8	88	-	-	-	-
2	-	-	15	80	15	80
4	14	79	14	100		
7	4	75				
10	8	75				
15	14	86				

Table 4. IMViC analysis of EC positive cultures isolated during storage of Manila clam shellstock

Storage time (days)	10 C		20 C		27.5 C	
	Total No. Isolates	% E.coli	Total No. Isolates	% E.coli	Total No. Isolates	% E.coli
0	64	89	64	89	64	89
1/2	-	-	46	87	35	91
1	19	95	-	-	-	-
2	-	-	28	89	25	100
4	39	90	43	88		
7	16	93				
10	15	100				
15	9	100				

Studies on Depuration-induced Changes in the Composition of the Pacific Oyster (*Crassostrea gigas*)

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ABSTRACT

Oysters are an important fisheries product in coastal areas of the United States. One of their principal market forms is as an iced product. The bacterial flora of the intestinal contents cannot be removed and becomes part of the final product. The number of fecal coliform bacteria can be reduced by depuration and thus reduce the potential hazard. This latter procedure could affect the quality of the final product. Since glycogen is important in the commercial value of the oyster, any loss of carbohydrate would be undesirable. Thus, a preliminary study on the effect of depuration on the composition of oysters was undertaken.

MATERIALS AND METHODS

Pacific oysters (*Crassostrea gigas*) of 7-10 cm. in length were obtained from the depuration unit of the U.S.P.H.S. Shellfish Sanitation Laboratory at Purdy, Washington. These oysters had been held for 1/2 to 26 days, while control groups had been held under refrigeration at 4-5 C for 1/2 to 3 days.

The samples were weighed into blendor jars and two volumes of water added and then blended at high speed for 1-1/2 minutes. This procedure failed to disintegrate the oysters completely. The procedure was modified, in that only one-half the water was added, the oysters blended for 1/2 min., and the balance, *i.e.* one volume of water, was added and the blending process continued for an additional 1 min. One ml. aliquots were pipetted into tared aluminum dishes and these dried at 105 C to constant weight.

The specific gravity of the homogenate was measured, using a pipet in which the weight of a given volume of oyster homogenate was compared to a given volume of distilled water at room temperature, approximately 20 C.

Glycogen was determined, using essentially the method of Good and Somogyi (1933), as modified by Dubois et al. (1956) and Montgomery(1957). One ml. of the homogenate was added to 2 ml. of 30% KOH, held in a boiling water bath, and the digestion continued until the samples cleared. 1.1 to 1.2 volumes of 95% ethanol was added to give the final concentration of 67-70%, in order to precipitate the glycogen. The samples were centrifuged and washed two times with additional volumes of 70% alcohol,

centrifuging and decanting the supernatant at each washing. Glycogen was determined colorimetrically, using the phenol-sulfuric acid method (Montgomery, 1957), and the results compared to glycogen standards prepared in a similar manner. The results were calculated on the basis of dry matter.

RESULTS AND DISCUSSION

The weight of the homogenate in grams/ml., as shown in Appendix, Table 1, reflects the dry matter present in the shellfish. Those which contained a very low proportion of dry matter had a specific gravity of about 1.0. As the dry matter increased, the specific gravity decreased within reasonable limits. The normal oyster would appear to have a specific gravity of approximately 0.90 to 0.95. The glycogen expressed as mg/g of dry matter reflected the holding conditions of the oysters. The controls refrigerated for 1/2 day and those depurated for 1/2 day had very low glycogen levels. There is no explanation for these low values, and the samples held 3 days returned to a normal value, possibly due to loss of moisture, as reflected in the dry matter. Holding oysters in the depuration facility did not cause appreciable change in glycogen levels during 21 days, except in those oysters which were supplied with a filtered water. In these the dry matter declined sharply and the glycogen increased proportionally. This change apparently reflected a simple increase in the amount of tissue water, and if the dry matter were corrected to 20%, the glycogen levels were more consistent. It is quite evident that the initial condition of the oyster will vary and that this condition will reflect the handling methods.

Table 1. Relationship of pre-conditioning of oysters to composition

Treatment	Days of treatment	No. in Sample	Weight of Sample g	Dry Matter g/100g	Glycogen mg/g dry matter	Glycogen 20% dry basis*	Weight of homogenate g/ml
Controls refrigerated at 4-5 C	1/2	3	114	16.2	86	70	0.90
	3	3	120	20.0	162	162	0.90
Depurated	1/2	3	108	15.7	134	105	0.91
	1	3	127	16.3	197	161	0.95
	3	3	65	11.7	297	174	0.92
	21	3	93	18.1	188	170	0.88
Starved	26	3	84	8.3	515	213	1.01

*Adjusted to 20% dry matter basis.

Studies on the Behavior of a Bacteriophage in the Pacific Oyster (Crassostrea gigas)

J. C. Hoff and W. J. Beck

INTRODUCTION

That shellfish ingest and concentrate bacteria present in the water during their feeding activities is well known. The public health implications of this phenomenon have been extensively studied.

Certain groups of viruses such as poliomyelitis, Coxsackie, ECHO, and infectious hepatitis virus are present in the human intestinal tract and therefore are possible pollutants of shellfish growing waters. It is of interest to know whether or not these very small biological particles (approximately one-fiftieth the size of Escherichia coli) are ingested, concentrated, and eliminated by shellfish in the same manner as bacteria and also to determine their ability to survive in shellfish under various conditions.

Because of a lack of equipment necessary for animal virus work and because of the difficulties in quantitative analysis of heterogeneous mixtures for these particles. a bacteriophage was used in these studies. The bacteriophage used, while larger than the enteroviruses is much smaller than Escherichia coli. A comparison of the relative sizes is given in Table 1. The shellfish species used in this study was the Pacific oyster (Crassostrea gigas).

MATERIALS AND METHODS

Bacteriophage. The phage was isolated from sewage using E. coli C2 as the host bacterium. Electron microscopy[1] showed that the phage was 390 mu long and 110 mu wide. Initial experiments showed that the phage was stable in sea water and was inactivated by ultraviolet irradiation.

Oysters. The oysters (Crassostrea gigas) were collected in upper Burley Lagoon. Usually oysters 10-15 cm long were used. Samples consisted of from 6 to 10 animals.

Media. Media and methods described in APHA Recommended Procedures for the Bacteriological Examination of Sea Water and Shellfish (Third Edition, 1962) were used for determination of E. coli C2 in samples except that the

[1] electron microphotographs were made by Mr. Dale Birdsell, Dept. of Bacteriology and Public Health, Washington State University, Pullman, Washington.

EC test was conducted at 46 C rather than 44.5 C. Media used for phage production and assay were those described by Groman and Suzuki (1962). Overlay agar consisted of 1.2% agar (Difco) in distilled water.

Blending procedure for phage assays. The conventional blending procedure caused destruction of phage. A modified procedure in which the samples were blended for 45 seconds at 8,000 RPM was used in preparing oyster meats for phage analysis.

Phage assay method. All samples were assayed in duplicate. One ml of the sample dilution to be assayed was placed in sterile cotton plugged tubes (13mm x 100mm) held in a water bath at 45 C. One ml of overlay agar, previously melted and cooled to 45 C was added. Then 0.1 ml of a 5-6 hour broth culture of E. coli C2 was added and the tubes were shaken briefly in the water bath. The contents of the tubes were then poured over the surface of freshly prepared phage assay agar plates and the plates were rotated to spread the overlay. After the overlay had hardened, the plates were inverted and incubated at 28 C for 48 hours. Plaques were counted using a Quebec colony counter.

Accumulation-elimination experiments. The procedures were similar to those used previously in bacterial accumulation-elimination experiments (Presnell, 1963). Water flow usually was adjusted to 9.6 liters/hr/oyster. In some of the experiments the flow rate/animal was reduced.

RESULTS

Development of blending process. The deleterious effect of the blending process on recovery of phage from blended suspensions is shown in Table 2. The results indicate that the loss resulted from the blending process itself since blended meats shaken with phage suspension showed no appreciable loss even after 30 minutes incubation at room temperature. However, when blending was used, either with or without oyster meats, losses of approximately 50 percent occurred.

In the experiments shown in Tables 3 and 4 sterile 0.1% peptone water was substituted for oyster meats. The effects of various blenders and blending times are shown in Table 2. Losses became progressively greater with extended blending time. Losses were smaller in Waring blendors than in the Oster blenders but in all cases definite losses occurred.

In subsequent experiments a rheostat was used to control blending speeds. Blender speeds at various rheostat settings were calibrated with an odometer. The results of varying blending speeds and times are shown in Table 4. In the Oster blender no losses occurred after 45 seconds at any of the three speeds but definite losses occurred after 90 and 180 seconds blending time. In the Waring blendor recovery was good at all

three speeds after 90 seconds blending time. The results of the same type of experiment using oyster meats in place of 0.1% peptone water are shown in Table 5. It was concluded that blending for 45-60 seconds at 8,000 RPM would give satisfactory phage recovery. Oster blenders with baffles produced more homogeneous preparations than Waring blendors at this speed because of more violent action.

Accumulation-elimination experiments. The results of the initial accumulation-elimination experiment are shown in Table 6. The phage concentration was higher in the oyster shell liquor than in the oyster meats after 42 hours of pollution. The presence of feces in the tank and the presence of phage in the shell liquor indicated that the oysters had been pumping during this period. This experiment was repeated with results similar to those shown in Table 6. In one of the repeated experiments, oyster feces and pseudofeces were examined for phage content. The results, shown in Table 7, indicate that phage particles were not concentrated either in feces or pseudofeces.

During the initial experiment water samples were collected and centrifuged at 3000 RPM for 40 minutes to determine whether the phages were freely suspended in the water or were attached to larger particulate matter which would be sedimented at this speed. The results of these analyses are shown in Table 8. No difference in phage concentration in supernatant and sediment fractions was found, indicating that the phages were not attached to larger particles. The centrifugation used is sufficient to sediment E. coli from culture media. When 20 ml samples of the water were filtered, no plaques were found. However, when previously filtered sea water was contaminated with phage and refiltered the phage was also retained by the filter. This retention was evidently due to physical factors rather than to attachment of the phage to larger particulate matter.

In further attempts to determine whether or not the oysters were filtering our phage particles, experiments employing a large number of animals held in tanks through which phage contaminated water was flowing at a low rate were designed. It was assumed that if the oysters were actively feeding and ingesting the phages the effluent water from the tank should contain lower concentrations of phage than the influent water. The results of several of these experiments are shown in Table 9. Consistent reductions in phage concentration in effluent samples compared with influent samples were not found. In experiments 3 and 4, oysters in a control flat were exposed under similar flow rate conditions to water polluted with E. coli. In experiment 3, the last three samplings show considerable difference between influent and effluent E. coli concentrations. In both experiments 3 and 4, E. coli was present in similar concentrations in both oyster meats and shell liquor, while no phage was found in the meats of the phage polluted oysters.

In several of the experiments, the oysters were allowed to pump in phage-free flowing sea water following the accumulation phase. The results of three of these experiments are shown in Table 10. The oysters became free of detectable phage in every case by the time the first sample was taken.

In several instances oysters which had been exposed to phage pollution were taken from the water at the end of the polluting period and stored dry at 5 C. Table 10 shows the effect of storage at this temperature on survival of the phage in oyster meats and shell liquor. The results indicate that phage was quite stable in both the meats and shell liquor at this temperature for at least 12 days.

DISCUSSION

The results of the initial accumulation experiments were not similar to results previously obtained with oysters using E. coli as a pollutant (Kelly et al, 1960), (Kelly, 1961). However, it was possible that the phage particles were being ingested and subsequently inactivated or held by some means that made them undetectable. The mucous of the oyster could possibly physically bind the particles or chemically inactivate them since many virus inhibitors are mucins or mucoproteins (Luria, 1953).

The series of experiments employing high oyster/water flow ratios were set up to test this possibility. However, the results of these studies are regarded as inconclusive because EC MPN's of effluent and influent water samples from the control tank polluted with E. coli did not differ consistently. Oyster meats did contain higher concentrations of E. coli than phage but again this may have been because the phage was held so as to be undetectable. The reason for the failure of the oysters to accumulate E. coli concentrations higher than those of the surrounding water is not known. It is possible that lack of oxygen or food because of the low flow rates caused the oysters to be inactive.

The results of Hedstrom and Lycke (1964), using poliovirus as a pollutant in a nonflowing system were similar to those found in this study. Shell liquor contained virus concentrations similar to that of the surrounding water while gill and mantle tissue and the remainder of the oyster body usually contained much lower concentrations.

Depuration of the phage-infected oysters proceeded rapidly with no phage being detectable after 18 hours. The results of Hedstrom and Lycke (1964) differed from this. They found that oysters transferred from infected to uninfected water showed little change in virus concentration after 24 hr. and that oysters transferred five times over a period of 100 hours still contained poliovirus. They concluded that the virus was associated with the oysters in such a way that it was not removed during these transfers.

The phage was stable in contaminated oyster shellstock stored at 5 C. Similarly, Hedstrom and Lycke (1964), found that poliovirus was stable for at least 56 hours in either 2 percent or 50 percent oyster tissue homogenate stored at 23 C.

It appears that neither phages nor poliovirus freely suspended in water are accumulated and concentrated to the same extent as E. coli. However, it is possible that they may be attached to larger particles and ingested in this way. The stability of these particles in oyster meats

and shell liquor is of considerable importance from the public health standpoint. This is enhanced by dosage response curve data which indicate that one virus particle is sufficient to initiate infection (Luria, 1953). On the other hand, with the exception of several outbreaks of infectious hepatitis (Roos, 1956; Mason and McLean, 1962), oysters have not been implicated in epidemics of other viral diseases.

The experiments employing large oyster/water flow rate conditions will be continued. Attempts will be made to improve conditions, e. g., oxygen supply, so that the animals will feed more actively. Oyster meats will be washed in phage-free water prior to examination in attempts to determine whether the phage is closely associated with the oyster meats or is found in the meats because of incomplete separation of meats and shell liquor.

COMMENTS BY PARTICIPANTS

Dr. Liston opened the discussion by commenting on the absorption of animal viruses as compared to phages. Mr. Hill and Dr. Hoff discussed the relative removal of E. coli and the phage used in the study. Dr. Dollar and Mr. Hill discussed methodology of isolation of phage. Dr. Dollar suggested the use of EDTA in connection with filters used. Dr. Quayle commented on the low level of accumulation of bacteria by the oysters in the latter part of the study. A brief discussion was held between Mr. Kelly and Dr. Liston on the use of other viruses as a test organism.

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Roos, B. 1956. Hepatitis epidemic conveyed by oysters. Svensk. Lakantidn. 53: 989-1003.

Table 1. Comparative sizes of E. coli bacteriophage C2 and some enteric viruses

Microorganism		Size (Millimicrons)
Escherichia coli	width	400 - 700
	length	1,000 - 4,000
Bacteriophage C2	width (at head)	110
	length (overall)	390
Poliomyelitis virus		27 - 30
Coxsackie		27 - 30
ECHO virus		20 - 90

* * * * * *

Table 2. Effect of blending process on recovery of phage from oyster meats

Treatment	Plaque Counts (%Loss or Gain)[a] Observer 1	Observer 2
None (control)	295 plaques	286 plaques
Meats blended then mixed with phage suspension and chaken	+ 4%	+ 7%
Above preparation allowed to stand at room temperature for 30 minutes, then sampled again	- 6%	- 1%
Meats blended with phage suspension 90 seconds at full speed (15,600RPM) in Oster blender	-62%	-58%
Same as above except 0.1% peptone water used in place of oyster meats	-40%	-39%

[a]Control equals 100%.

Table 3. Effects of various blenders and blending times on recovery of phage suspended in 0.1% peptone water

Type of Blender	Speed(RPM)	Blending Time (Seconds)	Plaque Counts (% Loss or Gain)[a]
None (control)			189 plaques
Pint Oster Blender	15,600	45	-28%
		90	-70%
Large Glass Waring Blendor	15,200	45	-16%
		90	-33%
Small Glass Waring Blendor	15,200	45	-17%
		90	-47%
Aluminum Waring Blendor	15,200	45	- 7%
		90	-25%

[a]Control equals 100%.

Table 4. Effects of blending speed and time on recovery of phage suspended in 0.1% peptone water

Type of Blender	Speed (RPM)	Blending Time (Seconds)	Plaque Counts (% Loss or Gain) [a] Observer 1	Observer 2
None (Control)			174 plaques	-
Pint Oster Blender	5,600	45	+ 5%	-
		90	-17%	-
		180	-20%	-
	8,000	45	- 5%	-
		90	-19%	-
		180	-20%	-
	12,000	45	+ 2%	-
		90	-26%	-
		180	-37%	-
None (Control)			173 plaques	171 plaques
Large Glass Waring Blendor	5,600	45	+21%	+18%
		90	+ 9%	+ 9%
		180	+16%	+16%
	7,500	45	-	+16%
		90	-	+19%
		180	-	+12%
	11,500	45	-	+ 8%
		90	-	+ 5%
		180	-	-11%

[a] Control equals 100%.

Table 5. Effect of blending speed and time on recovery of phage from oyster meats

Type of Blender	Speed (RPM)	Blending Time (Seconds)	Plaque Counts (% Loss or Gain) [a] Observer 1	Observer 2
None (Control)			110 plaques	100 plaques
Half Gallon Oster Blender	12,000	45	+ 13%	+ 15%
		90	-	-
		180	-	-
	8,000	45	+ 8%	+ 5%
		90	- 7%	- 2%
		180	-23%	- 20%
Large Glass Waring Blendor	11,500	45	- 7%	- 9%
		90	- 9%	- 6%
		180	-	-
	7,500	45	+ 5%	+ 6%
		90	-15%	+ 11%
			-22%	- 24%

[a] Control equals 100%.

Table 6. Accumulation and elimination of bacteriophage by Pacific oysters

		Plaque Counts/ml or g						
		Pollution Aquarium		Flat A			Flat C (Control)	
Date	Time	Start	Finish	Water	Oyster Meats	Oyster Liquor	Water	Oysters
3-25-64	2200						0	
	2215	100,000						
	2230			50				
3-26-64	0900						0	
	0930			780				
	1500		96,000	680	80[a]			
	1530	120,000						
3-27-64	1030						0	
	1100			520				
	1500		89,000	430			0	0[a]
	1600 -	Pollution stopped			15	190		
3-29-64	1030			0	0	0		

[a] Liquor not separated from meats.

* * * * *

Table 7. Phage concentration in oyster feces and pseudofeces

Duration of Pollution (days)	Water	Oyster Feces	Oyster Pseudofeces
1	460	520	620
2	640	740	780

Table 8. Effects of centrifugation and filtration on bacteriophage content of sea water

Sample	Plaque Count/ml			
	No Treatment	Centrifuged[a] Supernate	Sediment	20 ml Millipore Filtered[b]
Sea Water - Flat A 3-26-64	780	710	780	0
Sea Water - Flat A 3-27-64	520	540	560	0
Phage diluted in Millipore filtered sea water	1,000			0

[a] 3,000 RPM for 40 min.

[b] filter grids HA 0.45 u

Table 9. Accumulation of bacteriophage and Escherichia coli by Pacific oysters under high oyster/water flow ratio conditions

Exp. No.	No. of Oysters	Water Flow Rate (ml/min.)	Duration of Pollution (hours)	Phage plaques/ml or g				Escherichia coli MPN/ml or g			
				Water		Oysters		Water		Oysters	
				Influent	Effluent	Meat	Liquor	Influent	Effluent	Meat	Liquor
1	22	450	6	850	970						
			23	550	540						
			30	690	730						
			47	1,300	1,300	35	490				
2	38	80	3	680	760						
			19	590	530						
			20	610	390	15	290				
3	30 (Starved)[a]	250	23	120	113			7.9	33		
			26	85	92			13	33		
			44	87	122			1,300	350		
			49	116	81			3,300	1,300		
			68	81	97	0	-	330	130	230	79
4	33	250	14	126	127			790	1,300		
			18	130	142			1,300	1,300		
			23	93	101			790	1,100		
			35	111	125	0	33	3,300	1,700	330	490

[a]Oysters held at flow rate of 250 ml/min for approximately 40 days prior to experiment.

Table 10. Elimination of bacteriophage by Pacific oysters

Depuration Time (hours)	Phage plaques/ml or g		
	Water	Oyster Meats	Oyster Liquor
0	430	15	190
18	-	0	0
0	640	35	490
18		0	0
0	111	0	33
26		0	0

****

Table 11. Effect of dry storage at 5 C on bacteriophage concentration in Pacific oyster shellstock

Storage Time (days)	Phage plaques/ml or g		
	Water	Oyster Meats	Oyster Liquor
0	430	15	190
2	-	15	360
0	640	35	490
2	-	40	620
5	-	16	380
11	-	6	300
0	111	0	33
11	-	0	25
0	300	3	220
12	-	30	110

PART II PROPOSED RESEARCH ACTIVITIES FOR FISCAL YEAR 1965

PROPOSED RESEARCH ACTIVITIES FOR FY 1965

W. J. Beck

The following proposals were presented at the 1964 conference. An outline of each proposal was presented. Participants discussed each proposal and offered many excellent suggestions that augmented each proposal. In addition to formal presentations, several proposals were made from the floor.

OVERALL PLAN FOR 1965

A brief discussion of previous research, continued research, and projected research was rendered. Certain studies have now been completed. Among these are: 1) storage studies on shucked Pacific oysters; 2) storage studies on Pacific oyster and clam shellstock; 3) laboratory phase of accumulation-elimination of bacteria by oysters and clams and 4) phase 2 of the ecological study. All of these studies have either been published, or will be published within the very near future.

Studies to be continued through FY 1965 include: 1) accumulation and elimination of viruses by shellfish; 2) the investigation of oceanography as applied to shellfish growing areas.

New studies will include: 1) botulism; 2) pilot plant studies on depuration; 3) antimicrobial agents and 4) interrelationship of commercial organisms to indicators plus possibly pathogens.

Thus there has been a certain amount of change in the emphasis placed on the proposed research program of the Northwest Shellfish Sanitation Laboratory for FY 1965.

COMMENTS BY PARTICIPANTS

Mr. Girard asked about former proposals that seemed to have been lost. He was especially interested in research on wet storage. Mr. Beck stated that this study had only been tabled and would be made a part of the FY 1965 program.

Mr. Foster commented on the need for an evaluation of the relationship of coliform and fecal coliform in the environment. Mr. Beck suggested that part of the question would be forthcoming with the publication of the ecological study. Other background material may be found in recent publications.

Mr. Bowers described the renewed interest in Olympia oysters. The Olympia Oyster Growers Association has indicated a desire for storage studies similar to that performed on Pacific oysters and clams. Mr. Beck stated that the Northwest Shellfish Sanitation Laboratory would be most happy to cooperate with the Olympia Oyster Growers Association in this study.

PROPOSAL

Accumulation and elimination of enteroviruses in West Coast shellfish

The bactericidal efficiency of ultraviolet irradiation for the treatment of sea water used in shellfish depuration has been demonstrated. That ultraviolet irradiation inactivates viruses in nonabsorbent media such as water and salt solutions has also been shown. However, particulate matter and other organic compounds found in natural sea water may exert a protective screening effect. Therefore, the viricidal efficiency of U. V. irradiation for sea water under various experimental and natural conditions of turbidity and color should be determined.

A small scale U. V. treatment unit will be designed using the principles incorporated in the "Purdy unit". Water flow and U. V. intensity will be variable. The use of one representative each of the polio, ECHO, and Coxsackie virus groups and Escherichia coli bacteriophage C2 is anticipated. If the assay procedure permits, the pollutant pool will consist of equal concentrations (plaque forming units) of each virus. The contaminated sea water will contain approximately 1000 PFU/ml of each virus. Assays will be done by the plaque method.

After the amount of U. V. irradiation necessary for the destruction of viruses in deionized water at various flow rates has been determined, similar determinations will be made using sea water. The effects of both natural turbidity and turbidity produced by the addition of diatomaceous earth on lethality will be determined.

Studies will be continued on the accumulation, survival and elimination of viruses in shellfish. The present studies using a bacteriophage will be extended wherein the Sabin poliovirus will be used as the test virus to determine rates of accumulation, survival and elimination. After the proper cell lines have been established, assays for the Sabin poliovirus will be done by the plaque technic.

Pollution of a similar system with E. coli will be used as a control. Thus some indication of the activity of the test shellfish can be determined at the time of the accumulation-elimination of viruses experiments.

COMMENTS BY PARTICIPANTS

Dr. Bartsch and Mr. Girard requested information on how the test virus would be removed from the salt water system before discharge into the estuary. Mr. Beck explained the system of holding tanks and check samples that would be used to insure the safety of the salt water discharge.

Drs. Liston, Dollar and Hoff discussed the ramifications of the attachment of viruses to certain particle sizes. Dr. Berquist suggested the use of fluorescent microscopy in addition to the other methods for detection of certain virus.

Drs. Sparks, Liston and Hoff discussed the reasons why oyster tissues may or may not be applicable to the research in virology.

Mr. Kelly summed up the reasons for the need of an intensified study in the relationship of viruses to shellfish

PROPOSAL

Survival, outgrowth and toxin production from Gram-positive spore-forming bacteria

Although in recent years fish products from fresh and salt water sources have been implicated in outbreaks of botulism caused by Type E Clostridium botulinum, shellfish have not been associated in any way. Perhaps in the past the length of time shellfish have been held during and after processing may have eliminated the danger of toxin production. However, several factors would seem to make shellfish products a suitable vehicle for the production of this toxin. Among the factors are: a) Type E C. botulinum has been reported to be endemic in certain marine environments in the Pacific Northwest; b) with the combination of the feeding process of shellfish plus the viability of the microorganisms after harvesting potentially hazardous conditions could be established. It is, therefore, proposed that the following investigation be undertaken.

Attempts will be made to determine survival, outgrowth and toxin production of C. botulinum, Type E, in shellfish and other marine fauna from simulated commercial practices such as harvesting, cold processing, partial heat processing and frozen storage. Thus the factors needed to induce survival, outgrowth and toxin production of Type E C. botulinum in shellfish may be determined.

The competence of personnel at this station in working with C. botulinum has not been ascertained. In addition, the many ramifications of isolation and detection from shellfish and related biota must be predetermined. Therefore, the initial stage of this research will consist of adding a known spore suspension of Type E C. botulinum to shucked Pacific oysters.

Spores will be added at the rate of 1,000, 100 and 10 per gram of shucked combined oyster meats and liquors. Each lot of oysters will be divided in 17 aliquots of 12 oz. each, placed in commercial plastic containers and sealed. The containers will be divided into 4 lots and stored at -5 C, 3 C, 10 C and 20 C respectively. Each storage temperature will be sampled at periodic intervals according to data obtained from previous experiments on storage of shucked Pacific oysters.

Each aliquot tested will be homogenized in an evacuated blendor. The homogenate will be prepared as outlined in Examination of Foods for Enteropathogenic and Indicator Bacteria (PHS Pub. No. 1142) for isolation of spores and identification of toxin.

As technics are developed, certain modifications may aid in speeding the final results. In addition, as proficiency is increased, studies will be initiated for the presence or absence of toxin-producing Gram-positive spore-forming bacteria in terrestrial and marine environments.

COMMENTS BY PARTICIPANTS

Messrs. Bowers, Foster and Beck commented on the relationship of this research to industry. It was agreed that emphasis should be placed on the fact that no problem was present in the shellfish industry. This type of research would be used for preventive measures only. Mr. Beck explained the system of reporting that would be in effect.

Mr. Kelly questioned why oysters would be used as the initial animal in place of clams. The reason was only one of expediency in developing technics. It is hoped that clams would be included during the present study.

Dr. Craig explained the type of research that was being performed by his group and other interested researchers on the West Coast. Mr. Beck requested comments on the level of spores to be used. Dr. Craig considered these levels somewhat high. He suggested a level of 3 to 5 spores per gram of meat as being adequate. Dr. Craig also indicated that he had found excellent cooperation with all facets of industry in his studies.

PROPOSAL

Enteropathogenic E. coli

Studies will be continued on the relative significance of enteropathogenic E. coli as indicators of pollution. Screening tests using commercially available fluorescent antiserum will be made at regular intervals on E. coli isolated from sewage effluents, polluted soils, animal fecal material and streams tributary to a shellfish growing area. Final identification will be made by classical serological methods.

COMMENTS BY PARTICIPANTS

Dr. Berquist remarked on the inadequacies of present commercial antisera. Mr. Kelly suggested that perhaps a cooperative effort was needed to determine availability of antisera. Mr. Girard questioned why this study was limited to a shellfish growing area. Mr. Kelly noted that these organisms would only be used as a research tool. Mr. Michener questioned whether or not they would be found in sufficient numbers to be significant. Mr. Beck explained the general outline as suggested by Dr. Hosty in the State of Alabama. The general agreement was that a fairly low priority should be given this project.

PROPOSAL

Oceanographic methods

Methods developed by the physical oceanographer in FY64 will be continued on a seasonal basis to study current patterns, displacement, retention and other oceanographic factors related to Burley Lagoon. The data will be gathered by personnel at this station and analyzed through the cooperation of the physical oceanographer at the Northeast Shellfish Sanitation Research Center, Kingston, Rhode Island. Thus an investigation incorporating seasonal variations will be completed.

COMMENTS BY PARTICIPANTS

Dr. Quayle questioned the use of personnel that were not trained as oceanographers. He suggested the possibility of using a student oceanographer or working with other agencies in order that the proper interpretation be placed on the data gathered.

Mr. Foster suggested the use of tagged silt to cover flows etc. Mr. Girard objected to certain of these items in a commercial shellfish growing area. Mr. Kelly commented on the need to continue the study in order that the entire study on Burley Lagoon as a prototype could be completed. There is a need to correlate microbiology with oceanography. Mr. Beck was directed to contact the University of Washington to explore possibilities of assistance in this program.

PROPOSAL

Antimicrobial agents

Much interest in recent years has been given to certain antimicrobial agents that may be found in shellfish and other marine biota. With the introduction of cell cultures in virology, methods will be available to determine whether or not these antimicrobial agents are present in western species of shellfish. The extraction method of Prescott & Li will be used for purification of the antimicrobial agents. These extracts will be tested against both viruses and bacteria.

COMMENTS BY PARTICIPANTS

Dr. Dollar suggested several possibilities of technics available for separating materials according to molecular size rather than the simpler method of dialysis. Mr. Kelly commented on projects at other research centers and the increased interest shown in recent years.

PROPOSAL

Pilot Plant study on Depuration

In the past, research in depuration at this station has been maintained at the laboratory level. Interest of certain areas in this country has increased rapidly during the past several years to where the process must now be evaluated at a pilot plant level. Among the questions still to be answered are: loading capacity of tanks; design of equipment for cleanability, practicality and economic feasibility; the fate of certain indicator organisms during the depuration process as related to stacking, moving and water flow through the tanks; and whether or not recirculation of sea water may be used. In addition, as technics and laboratory scale depuration operations are completed in relationship to virology, these microorganisms will have to follow the research in bacteriology.

It is therefore proposed that a cooperative effort between a state agency, industry and the research center be initiated to investigate depuration on a commercial scale.

COMMENTS BY PARTICIPANTS

Dr. Sparks and Mr. Gruble discussed the part that industry might play in such a proposed study. Mr. Gruble felt that industry would look ahead and cooperate to make shellfish available for the study. Mr. Beck explained that the problem was one of disposal of the product rather than construction of the pilot plant.

Mr. Girard suggested that the potential problem would be one of clams rather than oysters and that clams should have priority over oysters.

Mr. Stonehouse commented on the problem in British Columbia. He then introduced Mr. Timothy,an oysterman from the British Columbia area. Mr. Timothy commented on the situation from the viewpoint of a grower.

Mr. Beck again asked whether or not industry, state agencies and the Northwest Shellfish Sanitation Laboratory could find a common purpose in planning such a study. Mr. Gruble stated that industry would look favorably upon the program if the PHS would initiate action.

Mr. Kelly outlined what the general concept as to size and objective of the pilot plant would be. Mr. Gruble's only concern was that of substituting depuration for the continued vigilance of protecting approved growing areas.

Mr. Kelly commented that we could not regard depuration for pollution abatement. Depuration would have to be regarded as a means of additional protection of the product.

Mr. Glude suggested that the study proceed as rapidly as possible. Ideally, specifications for an approved type of plant for industry use should be developed immediately.

Dr. Dollar and Mr. Kelly discussed the potentiality of using radioactive source as a substitute for ultraviolet treatment of the sea water. It was suggested that Mr. Beck contact Dr. Dollar in the very near future to discuss the feasibility of this idea.

Mr. Kelly concluded the discussion by commenting on various projects on the East Coast.

As there were no further comments on this or other proposed projects, Mr. Beck thanked all the participants for their comments and lively participation in the entire conference.

APPENDIX

AGENDA

Annual Shellfish Sanitation Planning Conference
September 14-15, 1964

MONDAY AFTERNOON	Sam Reed, Moderator
1:00 - 1:30	Opening comment on conference - Dr. Bernard Bucove
1:30 - 2:15	Storage studies on shucked Pacific oysters - J. C. Hoff
2:15 - 3:00	Effects of antifoaming agents and evacuation on bacteriological technics - T. H. Ericksen
3:00 - 3:15	COFFEE BREAK
3:15 - 4:00	Activities at other PHS research centers - C. B. Kelly
4:00 - 4:45	Oceanography report on Burley Lagoon - W. J. Beck
TUESDAY MORNING	Dr. John Liston, Moderator
9:00 - 9:45	Storage studies on Pacific oyster shellstock - J. C. Hoff
9:45 -10:30	Storage studies on hard-shell clam shellstock - T. H. Ericksen
10:30 -10:45	COFFEE BREAK
10:45 -11:30	Accumulation and elimination of bacteriophage in Pacific oysters - J. C. Hoff
11:30 - 12:30	Proposed activities for 1965 - W. J. Beck
12:30 - 1:30	NO HOST LUNCH
TUESDAY AFTERNOON	C. B. Kelly, Moderator
1:30 - 4:30	Proposed activities for 1965 - W. J. Beck

ATTENDANCE ROSTER

Federal Agencies

A. F. Bartsch	USPHS WS&PC	Portland, Oregon
P. N. Bardal	Dept. of National Health	Vancouver, B. C.
W. J. Beck	NW Shellfish Sanitation Lab.	Purdy, Washington
S. S. Copp	Dept. of National Health	Vancouver, B. C.
Geo. Dixon Lt. Col.	USA Veterinary Corps	Ft. Lewis, Wash.
T. H. Ericksen, Jr.	NW Shellfish Sanitation Lab.	Purdy, Washington
W. A. Felsing, Jr.	USPHS Region IX	San Francisco, Calif.
John Glude	Bureau of Comm. Fisheries	Seattle, Washington
R. W. Hill	Food & Drug Directorate	Vancouver, B. C.
J. C. Hoff	NW Shellfish Sanitation Lab.	Purdy, Washington
L. S. Houser	USDHEW PHS Shellfish Branch	Washington, D. C.
W. Jakubowski	NW Shellfish Sanitation Lab.	Purdy, Washington
C. B. Kelly	USDHEW PHS Shellfish Branch	Washington, D. C.
F. S. Kent	USPHS Region IX	San Francisco, Calif.
W. G. Kupp	U. S. Food & Drug Admin.	Seattle, Washington
L. C. Myers	NW Shellfish Sanitation Lab.	Purdy, Washington
R. W. Nelson	Bureau of Comm. Fisheries Technical Laboratory	Seattle, Washington
N. Neufeld	Dept. of Fisheries	Vancouver, B. C.
H. M. Risley	U. S. Food & Drug Admin.	Seattle, Washington
R. Speyrer 1st Lt.	USA Veterinary Corps	Ft. Lewis, Wash.
G. J. Vasconcelos	NW Shellfish Sanitation Lab.	Purdy, Washington
D. C. Zeiter 1st Lt.	USA Veterinary Corps	Ft. Lewis, Wash.

Industry

Bob Bower	Ellison Bros. Oyster Co.	Olympia, Washington
Earl Brenner	J. J. Brenner Oyster Co.	Olympia, Washington
Malcolm Edwards	Coast Oyster Company	So. Bend, Washington
Edward J. Gruble	Hilton Seafoods Co., Inc.	Seattle, Washington
Edward A. Timothy		Ladysmith, B. C.
Nat Waldrip	Pacific Coast Oyster Growers	Shelton, Washington

State Agencies

K. R. Berquist	Washington State Health Dept.	Seattle, Washington
T. P. Blair	Oregon State Health Dept.	Portland, Oregon
Bernard Bucove	Washington State Health Dept.	Olympia, Washington
H. B. Foster, Jr.	California State Health Dept.	Berkeley, Calif.
John Gerth	Washington State Health Dept.	Olympia, Washington
John Girard	Washington State Health Dept.	Olympia, Washington
Clarence V. Hall	Washington State Health Dept.	Seattle, Washington
Max G. Hays	Washington State Health Dept.	Olympia, Washington
Evelyn MacDonald	Washington State Health Dept.	Seattle, Washington
K. L. Michener	Oregon State Health Dept.	Portland, Oregon

State Agencies (Cont'd.)

Alfred T. Neale	Wash. Pollution Control Comm.	Olympia, Washington
Sam Reed	Washington State Health Dept.	Olympia, Washington
V. C. Reierson	Oregon State Health Dept.	Portland, Oregon
C. R. Stonehouse	Health Branch, Govt. of B. C.	Victoria, B. C.
R. Westley	State Shellfish Laboratory	Brinnon, Washington

Universities

James M. Craig	Oregon State University	Corvallis, Oregon
A. M. Dollar	College of Fisheries, U of W.	Seattle, Washington
Gary Houghtby	College of Fisheries, U of W.	Seattle, Washington
John Liston	College of Fisheries, U of W.	Seattle, Washington
Jack Matches	College of Fisheries, U of W.	Seattle, Washington
B. M. Slabyj	College of Fisheries, U of W.	Seattle, Washington
A. K. Sparks	College of Fisheries, U of W.	Seattle, Washington

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